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EXPLORING THE *IN VITRO* COMPARATIVE EFFICACY OF ANTIMICROBIAL AND ANTIOXIDANT POTENTIAL OF *NIGELLA SATIVA* AND *CROCUS SATIVUS*

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Abstract

Traditional medicinal plants are widely recognized as potential sources of bioactive compounds with antimicrobial properties. Among these, Nigella sativa (black seed) and Crocus sativus (saffron) have gained considerable attention for their therapeutic potential as alternative antimicrobial agents. This study aimed to evaluate the antimicrobial and antioxidant activities of ethanolic extracts of black seed and saffron against common pathogenic organisms, including Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Candida albicans. Bacterial strains were cultured on selective and differential media such as Nutrient Agar (NA), Eosin-Methylene Blue (EMB) Agar, Brain Heart Infusion (BHI) Agar, and Mueller-Hinton Agar, while C. albicans was cultured on Sabouraud Dextrose Agar. Antimicrobial activity was assessed using the disc diffusion method.

The ethanolic extract of black seed produced inhibition zones of 20 mm against S. aureus, 18 mm against E. coli, and 16 mm against P. aeruginosa, while C. albicans showed resistance. In contrast, the saffron extract demonstrated stronger antimicrobial activity, with inhibition zones of 26 mm against S. aureus, 24 mm against E. coli, 20 mm against P. aeruginosa, and 28 mm against C. albicans. Antioxidant activity was determined through the DPPH (2,2-diphenyl-1-picryl-hydrazyl) free radical scavenging assay, expressed as the concentration required for 50% inhibition (IC_{50}). The IC_{50} value of black seed extract was 10.2 ± 1.20 mg/mL at a concentration of 43.71 mg/mL, whereas saffron extract showed an IC_{50} value of 10.8 ± 1.02 mg/mL at a concentration of 0.5 mg/mL.

These findings suggest that saffron exhibits stronger antimicrobial and antioxidant potential compared to black seed, indicating its promise as a natural therapeutic agent.

Keywords: *Antimicrobial effects, Anti-oxidant, Extracts, Medicinal plants, Pathogenic bacteria*

INTRODUCTION

The rise of antimicrobial resistance (AMR) now threatens people and animals everywhere. Because many bacteria are exposed to antibiotics without cause and become resistant, a crisis in their effectiveness is being seen (1, 2). To solve this problem, we need to fully understand how resistance happens at the molecular and genetic levels. Looking at resistance through a biomedical lens, it has become common for bacteria, viruses, parasites and protozoa to develop resistance, along with cancer tumor cells (3). Bacterial strains create major health challenges as they can resist treatments and lead to lasting infections that are difficult to cure, decreasing the range of treatment options available (4).

To counter this shocking increase in antibiotic resistance, it is essential to seek and develop new alternative approaches. The identification and production of new antimicrobial agents and resistance-modulating agents can significantly contribute to slowing the development of resistance and maintaining the effectiveness of existing treatments (5). One such research avenue lies in finding bioactive agents that are natural products from sources such as plants, animals, and microbes, in order to design the future antimicrobial drugs (6).

An increasing amount of scientific evidence verifies the antimicrobial potential of plant compounds, and in particular, plants have shown to be an excellent source of bioactive molecules.

Although the global diversity of more than 300,000 plant species has yet to be fully explored, only a minimal percentage—roughly 15%—has been systematically evaluated for their pharmacological activity (7). Among these, *Nigella sativa*, or black seed, has come into limelight for its immense medicinal potential. Being a member of the Ranunculaceae family, this plant has been greatly prized in traditional medicine in various cultures for its medicinal effectiveness in the treatment of a variety of diseases (8, 9).

Recent scientific research has elucidated the immense pharmacological potential of *N. sativa*, relating its therapeutic effects to the existence of varied bioactive metabolites. Research indicates that the health-enhancing activities of *N. sativa* oil can be attributed to its high content of polyunsaturated fatty acids, Thymoquinone (TQ), phenolic compounds, and other natural antioxidants. Remarkably, *N. sativa* has about 26.6% oil content, of which 64.6% is linoleic acid (LA) and 20.4% is palmitic acid in its fatty acid composition, as documented in the earlier studies (10).

The scientific name for the saffron plant is *Crocus sativus*, a short perennial herbaceous plant under the family Iridaceous. It is commonly known to be a traditional medicine plant that is grown in almost all parts of the globe. The material known as saffron is formed from the dried stigmas of the plant and is mainly used in cooking and medicine. While saffron adds flavor and color to meals, it has a different value in the pharmaceutical sector as it calms and relieves pain when used to help with asthma, whooping cough and inflammation (11).

Saffron has been recognized for its therapeutic value, largely thanks to crocetin, crocins and other potent antioxidants that clean up harmful free radicals and control pro-inflammatory cytokines (12). Additionally, antimicrobial properties of *C. sativus* have been confirmed, particularly against common bacteria such as *S. aureus, E. coli,* and *P. aeruginosa* (13, 14).

The studies in literature points to more than 100 bio elements in saffron stigma, mainly consisting of flavonoids, terpenoids and anthraquinones (15-17). Because it contains many phytochemicals, it has seen wide use in medicine to treat breathing, stomach, menstrual and heart-related disorders (18). In addition, saffron has a number of pharmacological actions, including relaxing the body, helping with coughs and relieving stomach pains. Its antibacterial, antiseptic and antifungal capabilities are also seen (19, 20). What's more, saffron has been connected with antioxidant (21), anti-inflammatory (22), antihypertensive (23), antidepressant (24) and antitumor (25) effects. Current study objective is to evaluate the Antimicrobial and antioxidant potential of *Nigella sativa* and *Crocus sativus* and to compare their efficacy.

METHODOLOGY

PREPARATION OF *N. SATIVA* SEED AND *C. SATIVUS* EXTRACT

I got both *Nigella sativa* (Black seed) seeds and *Crocus sativus* (Saffron) stigma from an herbal store in Quetta, Balochistan, Pakistan. The extract was made by using the Solvent Extraction Technique. At first, the Black seed were thoroughly washed in water to clean them of dust, debris or other pollutants. Both Black seed and Saffron were left to air dry at the normal temperature of the area. Following drying, the samples were crushed into powder by an electric grinder.

All flasks were made by adding 5 grams of Black seed powder and 5 grams of Saffron powder, plus 50 mL ethanol and 50 mL of distilled water. I closed the flasks tightly with aluminum foil to ensure both contaminants and solvents were kept inside. The flasks were kept on a mechanical shaker and shaken uninterrupted for 48 hours to get the highest total extraction. Once the extraction process was done, all samples were centrifuged at 6000 rpm for 30 minutes, separated the liquid from the solid. The particles were collected by having the mixture pass through Whatman filter paper. The resulting filtrates were subsequently concentrated by evaporation under a rotary evaporator maintained at 45°C. The final extract concentrations of both Black seed and saffron were 10 ml after the rotary evaporation process. This process yielded crude ethanolic and aqueous extracts of Black seed and Saffron.

EVALUATION OF ANTIBACTERIAL ACTIVITY OF BLACK SEED AND SAFFRON *WELL DIFFUSION METHOD*

The antibacterial activity of *Nigella sativa* (black seed) and *Crocus sativus* (saffron) extracts against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa and Escherichia coli*) bacteria was assessed using the well diffusion technique (26, 27). Nutrient agar, EMB agar, Mueller-Hinton agar, Brain Heart Infusion agar, and Tryptic Soy Broth medium were set up according to standard procedure and sterilized using an autoclave at 121 °C for 20 minutes. Following sterilization, the media were left to cool before pouring into plates within a biosafety cabinet. The plates were placed in incubation at 37 °C for 24 hours to confirm sterility.

After confirmation of sterility, a bacterial lawn was grown on Mueller-Hinton agar (MHA) and Nutrient agar (NA) plates with the aid of a sterile cotton swab. Wells were punched into the agar with a sterile well borer (28), and ethanolic and aqueous black seed and saffron extracts were placed into the wells in order to determine bacterial sensitivity. The plates were incubated at 37 °C for 24 hours. Following incubation, the inhibition zones were recorded in millimeters (mm) as per (29, 30). Different antibiotic discs were utilized as positive controls, such as Ceftazidime + Clavulanic acid (CAL 40), Nalidixic Acid (NA-10µg), Furazolidone (FR-10µg), Tobramycin (ToB-10µg), Enrofloxacin (ENR-10µg), Lincomycin (MY-10µg), Cefotaxime (CTX-30µg), Streptomycin (S-10µg), and Oxytetracycline (OT-10µg). Dimethyl sulfoxide (DMSO) was used as the negative control.

SCREENING ANTIMICROBIAL ACTIVITY OF BLACK SEED, SAFFRON EXTRACTS AND ANTIBIOTICS

The Agar Well Diffusion Method was utilized to determine the antimicrobial potential of ethanolic and aqueous extracts of black seed and saffron, as well as some selected antibiotics. Three wells with a diameter of 8 mm on each agar plate inoculated with bacterial cultures were prepared using a sterile cork borer. The wells were assigned appropriately as "BSE", "SE", "+", and "-" to identify various test samples and controls.

A 100 µL aliquot of black seed and saffron extracts was gently pipetted into the wells designated as "BSE" and "SE", respectively. Moreover, antibiotic discs loaded with different antimicrobial agents were placed in the wells designated as "+" to determine their effectiveness. As a negative control, 100 µL of dimethyl sulfoxide (DMSO) was placed in the "-" wells so that any antimicrobial activity seen was due to the antibiotics or the extracts and not the solvent itself. The test was carried out with three bacterial cultures: *Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa*, each grown on individual agar plates. All the tests were carried out in triplicate to provide evidence of reproducibility and consistency.

After sample application, the inoculated agar plates were placed at 37°C for 24 hours for microbial growth and possible inhibition zone development. Upon incubation, the antimicrobial activity was evaluated using the measure of the clear zones around the wells. These inhibition zones reflected the degree to which the extracts or antibiotics inhibited the growth of bacteria, thereby proving their activity against the examined microbial strains.

DPPH RADICAL SCAVENGING ASSAY

Pure sample: 0.5mM in DMSO
Crude Sample: 0.5mg/ml in DMSO
DPPH (*Wako Chemicals* USA, Inc.) Solution: 0.3mM in Ethanol
The DPPH assay is typically run by the following procedure:

DPPH solution (95µl, 300µM) in Ethanol is combined with test solution (5µl, 500µM). The reaction is permitted to proceed for 30 min at 37ºC and absorbance is read using multiple reader, SpectraMax340 at 517 nm. After reduction, the color of the solution gets diminished (Violet to pale yellow) (31). Percent Radical Scavenging Activity (%RSA) is calculated by comparison with a DMSO containing control. The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as IC_{50} value. The IC_{50} values of compounds were calculated by using the EZ-Fit Enzyme kinetics software program (Perrella Scientific Inc. Amherst, MA, USA). N-acetyl cysteine, ascorbic acid and BHA are used as the reference compounds (32).

ANTIFUNGAL ACTIVITY OF BLACK SEED AND SAFFRON

Antifungal activity of extracts of *Nigella sativa* (black seed) and *Crocus sativa* (Saffron) was evaluated against *Candida albicans*. For this experiment, Sabouraud Dextrose Agar (SDA) medium was prepared and sterilized in an autoclave. After sterilization, sterility was checked for to ensure there was no microbial contamination prior to proceeding with inoculation. The fungal culture of *Candida albicans* was then inoculated onto the agar plates with a sterile cotton swab to ensure uniform distribution of the fungal culture. The inoculated plates were placed in incubator at a controlled temperature of 28 °C for 24 hours to provide sufficient growth of the fungus.

To assess the antifungal potential of *N. sativa* (black seed) and saffron extracts, well diffusion was used. This method involved making small wells on the surface of the inoculated agar plate very carefully. Then, 100 µ of black seed and saffron extracts were placed in those wells. The plates were then placed in an incubator. The antifungal potential of the extracts tested was assessed by reading the diameter of the inhibition zone that developed around the wells. The presence of a clear inhibition zone was proof of the extracts' ability to inhibit the growth of *Candida albicans*.

RESULTS AND DISCUSSION

The present investigation evaluated the antimicrobial potential of ethanolic extract of Black seed and saffron against three bacterial strains (*S. aureus, E. coli* and *P. aeruginosa*) and one yeast strain. The findings are overall consistent with previous research.

BLACK SEED EXTRACT

Black seed extract showed antibacterial activity against *Staphylococcus* spp., having clear zone of inhibition, whereas there was limited or no potential against *Pseudomonas aeruginosa* and *Escherichia coli* (Table I). All the findings in this context are in full accordance with earlier studies, in which selective efficacy of *N. Sativa* against Gram positive bacteria has been repeatedly reported .for example, it was indicated significant of inhibition for *S. aureus* using ethanolic extracts of *N. Sativa*, whereas potential towards *E. coli* and *Pseudomonas aeruginosa* was much weaker (33).

Table I. Antimicrobial activity of black seed's extract

Organism	Ethanolic extract (mm)	Aqueous extract (mm)	Antibiotics (mm)	Sensitive (S)/ Resistant (R)
Staphylococcus aureus	20	14	Ceftazidime+Clavulanicacid ≥ 18 mm Cefotaxime ≥ 23 mm	S / R
Escherichia coli	18	14	Ceftazidime+Clavulanicacid ≥ 18 mm Cefotaxime ≥ 23 mm	S / R
Pseudomonas aeruginosa	16	14	Nalidicxic Acid ≥ 19 mm Furazolidone ≥ 17 mm	R / R
Candida albicans	Nil	Nil	Nystatin	R / R

In the same way, mentioned effect of thymoquinone, the main active constituent of *Nigella sativa*, especially against *Staphylococcus aureus* but mentioned significantly lower activity against Gram-negative bacteria, which they attributed to the outer membrane barrier in Gram-negative organisms, which penetration of hydrophobic compounds (34).

The weak antifungal activity noted in this experiment also corresponds to earlier research. Although *Nigella sativa* has been documented to be moderately antifungal. Research indicates that its efficacy is inconsistent, varying with the species of fungi and concentration employed. It was noted that antifungal potential at higher concentrations, which could account for the fairly low antifungal potency at the concentration used in this experiment (35).

SAFFRON EXTRACT

Unlike Black seed, the saffron extract showed broad spectrum antimicrobial potential with large inhibition zones against all tested bacterial strains and strong antifungal potential at 100 µL concentration (Table II). According to earlier studies similar broad spectrum antimicrobial activities were reported. This is mostly because of the bioactive compounds of saffron such as crocin and crocetin that have both bactericidal and fungicidal effects (36, 37).

Table II. Antimicrobial activity of saffron's extract

Organism	Ethanolic extract (mm)	Aqueous extract (mm)	Antibiotics (mm)	Sensitive (S)/ Resistant (R)
Staphylococcus aureus	26	19	Ceftazidime+Clavulanicacid ≥ 18 mm Cefotaxime ≥ 23 mm	S / S
Escherichia coli	24	18	Ceftazidime+Clavulanicacid ≥ 18 mm Cefotaxime ≥ 23 mm	S / S
Pseudomonas aeruginosa	20	16	Nalidicxic Acid ≥ 19 mm Furazolidone ≥ 17 mm	S / S
Candida albicans	28	22	Nystatin	S / S

IC_{50} AND CONCENTRATION VARIABILITY

Black seed extract IC_{50} (10.2 ±1.20 mg/mL) validates its moderate antimicrobial activity. While activity of saffron extract at 0.5 mg/mL (10.8±1.02 mg/mL) indicates its higher antimicrobial potential (Table III).

Table III. Antioxidant activity of black seed and saffron

Sample Code	Conc. (mg/ml)	% Inhibition	IC50 ± SEM [µM]	% RSA (Radical scavenging activity)
Ethanolic Extract of Black Seed	43.71	83.1	10.2 ± 1.20 mg/ml	Active
Ethanolic Extract of Saffron	0.5	85	10.8 ± 1.02 mg/ml	Active
Gallic Acid (Standard)	0.5	95.3%	3.69 µg/ml	

Saffron had strong inhibitory action against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* when compared with the control antibiotics Ceftazidime + Clavulanic acid, Cefotaxime, Nalidixic Acid, and Furazolidone. This finding suggests that the antimicrobial potential of saffron is stronger than that of the control antibiotics. It could have alternate modes of action in inhibiting the test microbes. The black seed extract inhibited the growth of *Staphylococcus aureus*. Regarding the level of inhibition, the extract showed a high ability to inhibit bacterial growth (Fig. 1 and 2).

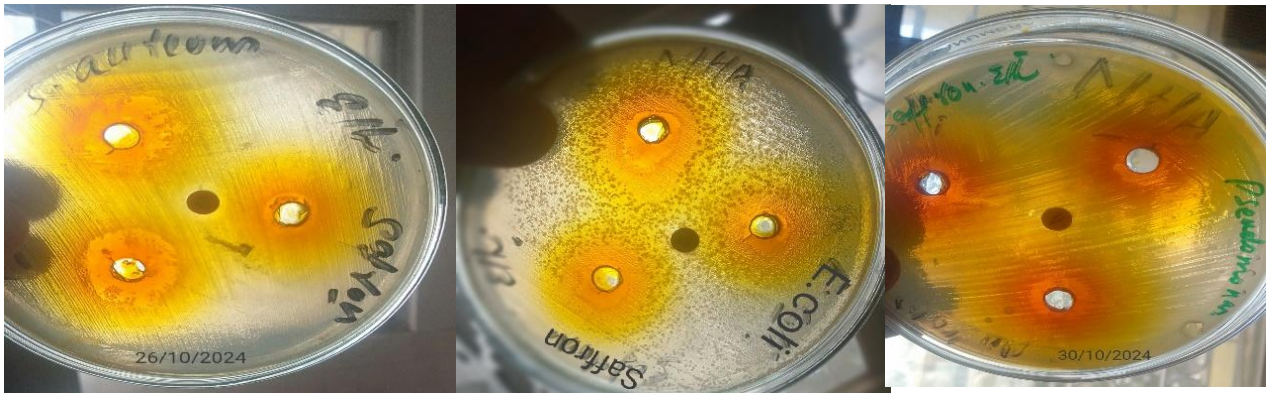

Fig. 1. Sensitivity testing showing clear zones on Mueller Hinton Agar

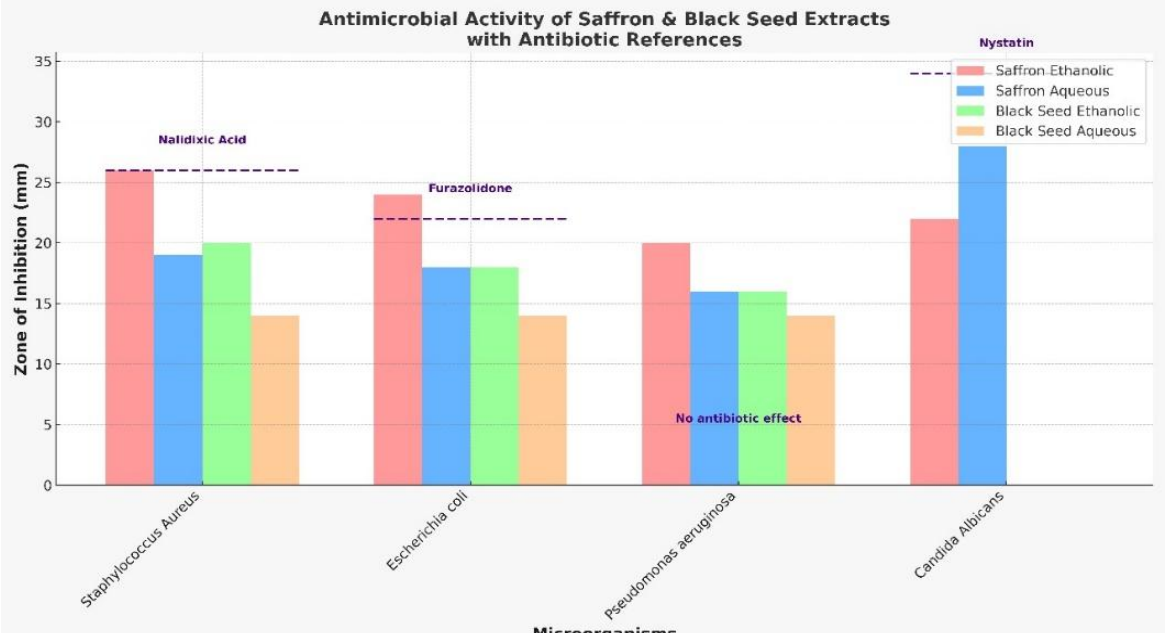

Fig. 2. Antimicrobial activity of saffron and black seed with reference antibiotics

Yet, none of the strains of either *E. coli* or *Pseudomonas aeruginosa* were susceptible to black seed extract. *E. coli* was sensitive to Cefotaxime, and *Pseudomonas aeruginosa* was sensitive to Nalidixic Acid and Furazolidone, as evidenced by clear zones of inhibition.

CONCLUSION

The research proves that saffron extract has broad-spectrum antimicrobial and antifungal potential, with strong inhibitory action against all the tested bacterial strains and the yeast strain. Black seed extract, on the other hand, had selective antibacterial potential, being effective against Staphylococcus species but not very effective against *E. coli* and *P aeruginosa*. Although black seed extract was measurably active at higher concentrations, saffron extract was active at the low concentration tested, suggesting that its activity might be dosage or extraction condition dependent. Saffron generally appears more active as a natural antimicrobial, whereas black seed could prove valuable for specific uses.

Recommendations:

To gain a better insight into the bioactive constituents that are behind the antimicrobial and antioxidant activities seen, extensive phytochemical analysis of *Nigella sativa* and *Crocus sativus* extracts must be carried out. This would help to pinpoint certain compounds (e.g., thymoquinone in black seed, crocin in saffron) that are accountable for their bioactivities.

In light of the growing concern over antibiotic resistance, the future research should assess the synergistic action of black seed and saffron extracts when combined with conventional antibiotics. This may open up the door for more efficacious combination therapies, particularly against resistant bacterial strains.

As a result of its selectivity, particularly against Gram-positive *Staphylococcus aureus,* black seed extract may be investigated in the development of targeted antimicrobial treatments, including skin infections or antiseptic topical.

Authors' contribution:

SR conceived the study & performed experiments; SAK supervised the overall project; PG assisted in antioxidant & antimicrobial assays; MS data collection & laboratory work; **YH** analyzed the data; MN literature review and IA assisted in proofreading and final editing.

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