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Research Article

# Antibacterial Activity of *Zingiber Officinale, Matricaria Chamomilla* and *Nigella Sativa* Extractions on the Growth of Pathogenic Bacteria Isolated from Different Clinical Specimens

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## Abstract

In this study, different concentrations of n-hexane, chloroform, ethyl acetate, methanol and aqueous extracts of the medicinal plants of *Zingebir officinal, Nigella sativa* and *Matricaria recutita*, were examined for their antibacterial activity utilizing the agar well diffusion technique against (Staphylococcus aureus, Enterococcus faecales, Echerichia coli, Klepsiella sp, Proteus sp, Pesudomonas sp, Salmonella sp, Serratia sp, Citrobacter sp). The results demonstrated that alcoholic extractions demonstrated astounding antibacterial. Phytochemical screening was directed for the best concentrates. The outcomes demonstrated the vicinity of a few distinctions in the constituents of the three unique plants tested. Ethiopian *Zingiber officinal* ethyl acetate extract contained alkaloids, anthraquinone glycosides, cumarins, flavonoids, saponins, tannins and triterpens. Ethiopian *Nigella Sativa* chloroform extract contained cumarins, flavonoids, triterpens and sterols. Sudanese Matricaria retutica methanolic extract contained alkaloids, anthraquinone glycosides, cumarins, flavonoids, saponins, tannins, triterpens and sterols. Cyanogenic glycosides were missing in the three extracts. Recent research has identified mentioned plants and their pure compounds which shown anti-microbial, and probably anti-inflammatory and other interesting biological activities.

**Keywords:** Antibacterial Activity; Pathogenic Bacteria; Medicinal Plants; *Zingebir officinal*; *Nigella Sativa*; *Matricaria recutita*; Phytochemical Screening

## Introduction

For a drawn out stretch of time, plants have been significant and vital wellsprings of natural products for the health of people, and they have an extraordinary potential for delivering new medications [1]. As indicated by the World Health Organization (WHO), plants are a wellspring of compounds that can battle disease, antimicrobial, antiviral and antifungal exercises [2]. Medicinal plants are critical substances for the investigation of their conventional uses through the confirmation of the pharmacological impacts and can be natural composite sources that act as new anti-infective agents [3]. Extensive variety of medicinal plant parts used to concentrate as crude medications and they have shifted medicinal properties. The distinctive parts utilized incorporate roots, stems, flowers, fruits, twigs exudes and modified plants [4]. Then again anti-infection resistance has turn into an overall issue. Behavioral variables, especially the misuse of antibiotics; and absence of contamination control practices in communities are the most widely recognized elements which prompt increment in antibiotic resistance [5]. These days, around 70 percent of the bacteria that cause diseases in hospitals are resistant to no less than one of the medications most usually utilized for treatment [6]. One approach to anticipate antibiotic resistance of pathogenic species is by utilizing new exacerbates that are not taking into account existing synthetic antimicrobial agents [7].

Therefore, moves must be made to lessen this issue, for instance, to control the utilization of antibiotics, create research to better understanding of the hereditary mechanisms of resistance, and to proceed with studies to grow new medications, either synthetic or natural. A definitive objective is to offer appropriate and effective antimicrobial medications to the patient [8].

## Materials and Methods

### Collection of specimen and isolation of the pathogenic bacteria

Under aseptic condition and using the specific agar media and techniques of bacterial identification, the pathogens were isolated from different clinical specimens and preserved for testing their susceptibility to the plant extracts [9,10].

### Sensitivity test

Depending on the Kirby-Bauer test, known as the disk-diffusion method, the sensitivity of the isolated pathogens to the following antimicrobial agents were tested: gentamycin (10 mcg), ampicillin (10 mcg), erythromycin (15 mcg) and tetracycline (30 mcg).

### Collection and preparation of plant samples

The selected plant for this work were *Nigella Sativa, Zingiber officinal* and *Matricaria recutita*, they were obtained from Al-Sooq Al-Arabi in Khartoum. Each sample imported from different sources, Syrian *Nigella Sativa,* Ethiopian *Nigella Sativa,* Nigerian *Zingiber officinal*, Ethiopian *Zingebir officinal*, Sudanese *Matricaria recutita* and Egyptian *Matricaria recutita*. The dried plant samples were cleaned from dust and grass then they were separately crushed to a powder form using sterilized mortar and pestle.

### Soxhlet extraction

Coming out concentrates in diverse solvents were evaporated and concentrated utilizing the rotational evaporator at 50C. Toward the end of this work there was 24 unique sorts of extractions were acquired. Every kind of concentrate was dissolved in dimethyl sulphoxid (DMSO 7%) and five diverse concentration were prepared 200%, 100%, half, 25% and 12.5% for testing their antibacterial activity [11].

### Aqueous extraction

Water extraction arranged by including 100 ml hot sanitized distilled water into 25 g of the grind materials of Nigella sativa and Zingebir officinal while 25 g of the crushed materials of Matricaria recutita required 250 ml hot sterilized distilled water, then were blended well until they got to be cold, then were separated utilizing 8 layers of muslin clothes, the filtrates were evaporated and concentrated to dryness utilizing the water bath at 100C. Utilizing hot sterilized distilled water three distinct concentrations were prepared, 100%, 50% and 25% and were examined instantly for their antibacterial activity.

### Screening the antibacterial activity of the plant extracts

Utilizing a sterile cotton swab, the suspensions of the chose bacterial strains were swabbed on the surface of sterile Mueler-Hiton Agar agar plates. Agar wells were readied with the assistance of disinfected cork borer number 4, utilizing a micropipette, 100 microliters of diverse concentrations of the plant extracts (200%, 100%, half, 25% and 12.5%) were added to different numbered wells in the plate. The plates were incubated in an upright position at 37C for 24 hours. The diameter of inhibition zones was measured in mm and the outcomes were recorded. The plant extracts which have a wide inhibition zone at the concentration 12.5% were tested for the antibacterial activity at less or smaller concentrations 6.0% and 3.0%.

### Phytochemical screening

General phytochemical screening for the active constituents was carried out for the most effective extracts using methods described by Martinez and Valencia, [12] with few modifications.

### Thin layer chromatography (TLC)

TLC was carried out according to Stahl [13], pre-coated TLC plate (Silica Gel $HF_{254}$). as stationary phase, chloroform: Methanol (80: 20), Toluene: Formic acid: Ethyl acetate (4: 1: 5), Toluene: Ethyl acetate (93: 7) Chloroform: Ethyl acetate (1: 1) as mobile phases.

## Results and Discussion

The zone of inhibition delivered by the extracts against the test organisms was recorded as a measure of antimicrobial action of the plant extracts, the outcomes demonstrated that the aqueous extracts of all the examined medicinal plants had not any activity against all the tried pathogenic bacteria these outcomes concurred with that acquired by Onyeagba., *et al*, [1] and Malu., *et al*, [14]. However, negative results don't demonstrate the absence of bioactive constituents, nor that the plant is inactive, since dynamic compound(s) may be present in inadequate amounts in the crude

extracts to show activity with the dose levels utilized [15]. Alcoholic plant extractions results indicated noteworthy antibacterial action.

All the plant extracts from Ethiopian *Zingiber officinal* (Table 1) showed activity against one or more bacteria tested. The best inhibition was seen in the chloroform extract as 23mm in diameter *against P. mirabilis* and 19mm against *Salmonella* at a concentration of 200 mg/ml compared with all antibiotics tested. Ethyl acetate extract showed wider activity in terms of the number of bacteria which were sensitive to the extract. The best inhibition zone was 20mm at a concentration of 200mg/ml and 17mm at a concentration of 100mg/ml against *S. aureus* compared with all antibiotics tested. Ethyl acetate extract showed inhibition zone of 12mm at a concentration of 100mg/ml and 14mm at a concentration of 200mg/ml against *P. mirabilis* compared with all antibiotics tested, which did not have any effect on *P. mirabilis*. Minimum inhibitory concentration was reported at concentration of 25mg/ml of ethyl acetate extract against *S. aureus* and *P. mirabilis.* Methanolic and *n*-hexane extracts showed less effect compared with all antibiotics tested except ampicillin, these results agreed with that obtained by Malu., *et al.* [14], Nalbantsoy., *et al.* [16] and Yousufi [17], with certain differences in the details of the study, such as the type of antibiotic or organic solvent.

| **Ext. con. mg/ml** | **Means of inhibition zone including well diameter measured in mm** | | | | | **Means of inhibition zone including antibiotic disc diameter measured in mm** | | | |
|---|---|---|---|---|---|---|---|---|---|
| **The sample** | 200 | 100 | 50 | 25 | 12.5 | Amp10 | Gen10 | TE30 | Ery15 |
| *K. pneumonia* (S1) | 9 | _ | _ | _ | _ | _ | 18 | 21 | 10 |
| *E. durance* (Sp 33) | 13 | 10 | _ | _ | _ | _ | 24 | 33 | 23 |
| *Staphylococcus aureus* (W 17) | 11 | 8 | _ | _ | _ | _ | 9 | 13 | 30 |
| *Staphylococcus aureus* (W 10) | 20 | 17 | 10 | 9 | _ | _ | 19 | 9 | 13 |
| *Staphylococcus xylosus* (W 19) | 13 | 11 | 7 | _ | _ | 11 | 23 | 16 | 30 |
| *P. mirabilis* (U 21) | 14 | 11 | 10 | 9 | _ | _ | 16 | _ | 10.5 |
| *P. mirabilis* (W11) | 14 | 12 | _ | _ | _ | _ | _ | _ | _ |
| *Pseu. Luteola* (Sp5) | 7 | 7 | _ | _ | _ | 25 | 32 | 42 | 40 |
| *Steno. maltiphilia* (Sp 11) | 9.5 | 7 | _ | _ | _ | 24 | 33 | 44 | 39 |

**Table 1:** Antibacterial activity of Ethiopian *Zingiber officinal* ethyl acetate extract (EZEE)

Sp = Sputum, S = Stool, W = Wound, U = Urine, The Numbers = Numbers of the Samples.

In case of Nigeria *Zingiber officinal* all plant extracts showed less antibacterial activity compared with antibiotics tested except ampicillin. The best inhibition zones were 23mm at a concentration of 200mg/ml, and 22mm at concentration of 100mg/ml and 20mm at a concentration of 50mg/ml of methanolic extract against *P. mirabilis* compared with all antibiotics tested. Minimum inhibitory concentration was reported at 12.5mg/ml of *n*-hexane extract against *E. durance* of 8mm inhibition zone, the same result obtained by Akintobi [18], with simple differences, whereas Sebiomo., *et al*, [19], did not agreed these results.

In case of Syrian *Nigella sativan*-hexane extract showed remarkable activity, the minimum zones of inhibition that reported were of 24mm against *E. durance and* of 18mm against *E. faecales* at concentration of 25mg/ml and of 11.5mm at 12.5mg/ml against *E. Facials* compared with ampicillin, gentamycin, and tetracycline.

Ethyl acetate extract showed minimum inhibition zone of 7mm at a concentration of 12.5mg/ml against *Salmonella* compared with all antibiotics tested, which did not have any effect against the bacteria mentioned except gentamycin, also ethyl acetate extract showed activity against *Serratia* with minimum zone of inhibition of 11mm at a concentration of 12.5mg/ml. *Staph. aureus* was more sensitive to ethyl acetate extract with a zone of inhibition of 11.5mm at a concentration of 100mg/ml than to gentamycin 9mm and ampicillin. Chloroform extract was active only against *Steno. maltiphilia* whereas methanolic concentrate did not demonstrate any activity against any bacteria examined.

Ethiopian *Nigella Sativa* extracts (Table 2) were more active than Syrian *Nigella sativa* extracts except the methanolic extract which gave similar results. The *n*-hexane extract demonstrated very large antibacterial activity against *E. durance, E. faecales, Steno. maltiphilia, Staph. xylosus* and *Serratia* at high concentrations compared with all antibiotics tested. A minimum zone of inhibition of 11.5mm was reported in concentration of 3mg/ml against *Serratia,* and inhibition zone of 9mm, at concentration of 6mg/ml against *E. durance* and 10mm inhibition zone with concentration of12.5mg/ml against *Steno. maltiphilia* and 13mm as zone diameter against *Staph. xylosus* and *E. faecales* 23mm inhibition zone. The chloroform

| Ext.con. mg/ml | Means of inhibition zone including well diameter measured in mm | | | | | Means of inhibition zone including antibiotic disc diameter measured in mm | | | |
|---|---|---|---|---|---|---|---|---|---|
| The sample | 200 | 100 | 50 | 25 | 12.5 | Amp10 | Gen10 | TE 30 | Ery 15 |
| *E. durance* (Sp 33) | 20 | 17 | 13.5 | _ | _ | _ | 24 | 33 | 23 |
| *E. faecales* (Sp 28) | 17 | 15 | 12 | _ | _ | _ | 25 | 33 | 21 |
| *Pseu. luteola* (Sp 4) | 25 | 25 | 20 | 13 | _ | 25 | 33 | 38 | 36 |
| *Pseu. luteola* (Sp 5) | 22 | 17.5 | 13.5 | 11.5 | _ | 25 | 32 | 42 | 40 |
| *Steno. maltiphilia* (Sp 11) | 23 | 16.5 | 15 | 10 | _ | 24 | 33 | 44 | 39 |
| *Staphylococcus aureus* (W 17) | 17 | _ | _ | _ | _ | _ | 9 | 13 | 30 |
| *Staphylococcus xylosus* (W19) | 23 | _ | _ | _ | _ | 11 | 23 | 16 | 30 |

**Table 2:** Antibacterial activity of Ethiopian *Nigella Sativa*chloro form extract (ENCE).

Sp = Sputum, S = Stool, W = Wound, U = Urine, A = Abscess, The Numbers = Numbers of the Samples.

extract showed antibacterial activity against 7 organisms belong to three different genes. The best result was 17mm against *Staph. aureus* and 23mm against *Staph. xylosus* at 200mg/ml compared with antibiotics tested except erythromycin, minimum inhibitory concentration was 25mg/ml. Ethyl acetate extract demonstrated antibacterial activity only at high concentrations. Approach results were acquired from a study reported by Yasni., *et al* [20], whereas Khalid., *et al* [21], and many other researchers inferred that the *Nigella sativa* methanolic extract indicated remarkable antibacterial activity against different pathogens.

The *n*-hexane extract of Egyptian *Matricaria recutita* demonstrated wider activity in terms of bacteria number, minimum inhibitory concentration was 12.5mg/ml, the best result was reported against *E. faecales* and *E. coli* compared with antibiotics tested except gentamycin. Ethyl acetate extract indicated the best antibacterial activity against *Staph. aureus*, minimum inhibitory concentration was 6mg/ml, the methanolic extract showed astounding results against *E. faecales* compared to all antibiotics tested. Chloroform extract indicated activity only at high concentrations.

The Sudanese *Matricaria recutita* showed wider activity in terms of bacteria number (Table 3) compared with Egyptian *Matricaria recutita*, minimum inhibitory concentration of methanolic extract was 50mg/ml, the best result was reported against *P. mirabilis,* 16mm at 200mg/ml and 11mm at 100mg\ml, compared with antibiotics tested which had not any effect. Generally *n*-hexane, ethyl acetate and chloroform extracts showed antibacterial activity at high concentrations. Ethyl acetate extract gave the best result against *S. aureus* and *P. mirabilis*, when chloroform extract showed activity against *S. aureus* and *Salmonella* compared antibiotics tested, Mawlood [22], Alireza [23], and Shakib., *et al* [24], and many studies agreed these results besides there were differences in the materials and methods.

| Ext.con. mg/ml | Means of inhibition zone including well diameter measured in mm | | | | | Means of inhibition zone including antibiotic disc diameter measured in mm | | | |
|---|---|---|---|---|---|---|---|---|---|
| The sample | 200 | 100 | 50 | 25 | 12.5 | Amp10 | Gen10 | TE30 | Ery15 |
| *E.durance* (Sp 33) | 13 | 10 | _ | _ | _ | _ | 24 | 33 | 23 |
| *E. faecales* (Sp 28) | 12 | _ | _ | _ | _ | _ | 25 | 33 | 21 |
| *E. faecales* (W 3) | 13 | 9.5 | _ | _ | _ | 19 | 32 | 37 | 27 |
| *E. faecales* (W14) | 14 | 11 | _ | _ | _ | _ | _ | _ | 17 |
| *Staphylococcus aureus* (W17) | 19 | 14 | 11.5 | _ | _ | _ | 9 | 13 | 30 |
| *Staphylococcus aureus* (W19) | 9 | _ | _ | _ | _ | 11 | 23 | 16 | 30 |
| *Salmonella* (S7) | 13 | 10 | 8 | _ | _ | _ | 20 | _ | _ |
| *Pseud. maltiphilia* (Sp11) | 8 | _ | _ | _ | _ | 24 | 33 | 44 | 39 |
| *Providensia alcalifaciens* | 12 | 8 | _ | _ | _ | _ | 22 | _ | 9 |
| *Serratia* (U11) | 12 | _ | _ | _ | _ | | 30 | 13 | 14 |
| *Pro. mirabilis* (S1) | 13 | 8 | _ | _ | _ | _ | 21 | 10 | 10 |
| *Pro. mirabilis* (W11) | 16 | 11 | _ | _ | _ | _ | _ | _ | _ |
| *Pro mirabilis* (Sp 18) | 13 | 8 | _ | _ | _ | 9 | 23 | _ | _ |

**Table 3:** Antibacterial activity of Sudanese *Matricaria recutita* methanolic extract (SMME**).**

Sp = Sputum, S = Stool, W = Wound, U = Urine, A = Abscess, The Numbers = Numbers of the Samples.

The results of the phytochemical screening of the most effective medicinal plant extract (Table 4) showed presence of some differences in the constituents of the three different plants tested. Ethiopian *Zingiber officinal* ethyl acetate extract contained alkaloids, anthraquinone glycosides, cumarins, flavonoids, saponins, tannins and triterpens. Ethiopian *Nigella Sativa* chloroform extract contained cumarins, flavonoids, triterpens and sterols. Sudanese *Matricaria retutica* methanolic extract contained

| Sample | Ethiopian *Zingiber officinal* ethyl acetate extract | | Ethiopian *Nigella Sativa* chloroform extract | | Sudanese *Matricaria recutita* methanolic extract | |
|---|---|---|---|---|---|---|
| **Test** | Observation | Results | Observation | Results | Observation | Results |
| Alkaloids | Turbidity | + | _ | _ | Turbidity | + |
| Anthraquinone glycosides | Blue- green colour | ++ | - | - | Blue- green colour | +++ |
| Cumarins | UV absorption | +++ | UV absorption | +++ | UV absorption | +++ |
| Cyanogenic glycosides | - | - | - | - | - | - |
| Flavonoids | Yellow colour | +++ | Yellow colour | + | Yellow colour | +++ |
| Saponins | Foam | + | - | - | Foam | ++ |
| Tannins | Blue- green colour | ++ | - | - | Blue- green colour | +++ |
| Triterpens | Purple colour | ++++ | Purple colour | ++++ | Purple colour | ++++ |
| Sterols | - | - | Green colour | + | Green colour | ++ |

**Table 4:** The phytochemical screening of the most effective medicinal plant extract results.

- = absence, + = weak presence, ++ = moderate presence, +++ = active presence, ++++ = more active presence

alkaloids, anthraquinone glycosides, cumarins, flavonoids, saponins, tannins, triterpens and sterols. Cyanogenic glycosides were absent in the three extracts. Recent research has identified mentioned plants and their pure compounds which shown anti-microbial, and probably anti-inflammatory and other interesting biological activities as described by Mawlood [23]; kamal., *et al* [25]; Singh and Singh, [26]; Alnahdi., *et al*, [27]; Kensa [28]; Vázquez., *et al*, [29]; Bhargava., *et al*, [30].

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## Conclusion

In the light of the results of the present study, we can infer that the examined plant extracts demonstrated awesome potential as antimicrobial compounds against pathogens contrasted with pure synthetic antibiotics. Accordingly, they can be utilized as a part of the treatment of infectious diseases brought about by resistant pathogenic bacteria.

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**Volume 3 Issue 5 May 2019**