Available online on 15.06.2024 at http://ajprd.com

Asian Journal of Pharmaceutical Research and Development

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Research Article

Comparative Antibacterial and Antifungal Studies with Different Plant Extracts

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A B S T R A C T

In the present scenario, resistance of pathogenic microorganisms to the most of antimicrobial (antibacterial and antifungal) agents is increasing. In this study we have compared the antibacterial and antifungal effect of some medicinal plants like neem, turmeric and ginger. The methanol extracts of these plants were prepared for determining their anti-bacterial and anti-fungal activity. Then comparative analysis of antibacterial effects between those extracts were tested in *E. Coli* and *Bacillus subtilis* culture. Also, the antifungal activity of these extractswas tested and compared using *Saccharomyces cerevisiae* culture. The antibacterial and antifungal activity was investigated using well diffusion method. All of the extracts have shown antibacterial and antifungal activity. The methanolic herbal extracts of ginger, turmeric rhizomes and neem leaves exhibit promising antibacterial properties against *E. Coli* and *Bacillus subtilis* and antifungal properties against *Saccharomyces cerevisiae*.

Key words: Antibacterial, antifungal, multidrug resistance, pathogenic microbes, synergistic effect

A R T I C L E I N F O: Received12 Jan 2024; Review Complete 15 April 2024; Accepted 16 May 2024; Available online 15 June. 2024

Cite this article as:
Mukherjee J, Varshitha P, Shruthi P, Sanjana N, Rao TR, Comparative Antibacterial and Antifungal Studies with Different Plant Extracts, Asian Journal of Pharmaceutical Research and Development. 2024; 12(3):50-57 DOI: http://dx.doi.org/10.22270/ajprd.v12i3.1395

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INTRODUCTION

Since primitive times, man has gone in different ways to search for cures and relief from various diseases by using several plants, plant products and plant-derived products. Medicinal plants are essential to maintainour health. Antibiotics are extremely dangerous to host organs, tissues and cells when used to treat illnesses. Herbs can be used to avoid the toxicity that antimicrobial drugs create [1]. Neem, ginger and turmeric are utilised in traditional medicine. The aim of the study to perform the comparative analysis of the antibacterial and antifungal properties of methanolic extracts from a portion of these plants.

One of the greatest accomplishments of contemporary science is the development of antibiotics to tackle with the microbial infections. The main concerns could be the development of resistance of pathogenic bacteria to currently available antibiotics [2].Infectious diseases are the reasons for the rising rates of mortality and morbidity in developing countries. A significant risk is the establishment of multi-drug resistance bacteria brought on by the incorrect use of broad-spectrum drugs. Plants with medicinal value that are abundant in secondary metabolites that have therapeutic qualities are being investigated as possible safer and more effective substitutes for manufactured medications. Different parts of plant such as roots, leaves, stem, fruits contain bioactive compounds and are potent sources of antimicrobial compounds [3,4]. Therefore, research into physiologically active compounds derived from plant species commonly used in herbal medicine is becoming increasingly relevant. These components might offer a potent new source of antibacterial action [5].

Pathogenic fungal infections are becoming more widely acknowledged as a growing concern to public health. The immunocompromised population, which includes cancer and

HIV patients as well as organ transplant recipients, has grown in recent years, which has led to an increase in the incidence of fungal infections. These issues are also linked to antibiotic resistance and toxicity when using many antifungal medications over an extended period of time. Furthermore, it has been known for a number of years that fungi are becoming resistant to the majority of medications. Many of the secondary metabolites that plants generate have antifungal properties. Comparably, a wide variety of plant extracts have been used in traditional medicine to treat fungal infections. Given that plants may produce defensive mechanisms against fungi, they seem like a promising source of antifungal chemicals [6].

There is a large range in the effectiveness of different herbal extracts against viruses, bacteria, fungiand parasites. Unlike other antibiotics that are designed to target particular types of bacteria, several herbs are effective against a broader variety of diseases. Rather of containing a single active component, herbal extracts are made up of several different molecules. This complexity poses a greater challenge for pathogens to develop resistance. Conversely, pharmaceutical antibiotics frequently comprise isolated chemical constituents, which bacteria can more readily adapt to and counteract [7]. Since herbal extracts often result in less side effects than antibiotics, they are frequently seen as a safer alternative. It is crucial to understand that many herbal medicines might potentially have adverse effects.Herbal medicine is unique in that it treats the patient as a whole, treating all symptoms as well as the underlying cause of illness.Herbal treatment aims primarily to improve general well-being and restore equilibrium.

Herbal remedies are preferred over synthetic antibiotics since they are less likely to have side effects. Our paper includes the work done to determine the antibacterial and antifungal activity of turmeric rhizomes, ginger rhizomes and neem leaves.

Ginger, scientifically known as Zingiber officinale. It belongs to family Zingiberaceae.Ginger is a well-known plant used for flavouring and medicinal purposes for many years. It is commonly used to treat health issues like pain, nausea, and vomiting. Research has shown that ginger has antiemetic effects in various conditions. Over 100 compounds have been found in ginger, with gingerols being the most important. Ginger has many biological activities, including antioxidant and antimicrobial properties. Recently, ginger has also been studied for its potential in treating cancer, chemotherapy-induced nausea, fatigue, and improving quality of life [8,9].

Figure 1: *Zingiber officinale*

Turmeric, also known as *Curcuma longa*, is a plant that grows naturally in the forests of South Asia and Southeast Asia, particularly in southern India. It belongs to Zingiberaceae family. Different parts of the turmeric plant are utilized for various purposes. The rhizomes are especially valuable due to their unique biological properties, colour, and scent. The leaves of turmeric serve as protective covers for food while cooking. Turmeric is composed of volatile and non-volatile compounds. Volatile compounds are extracted from raw materials like essential oils, while non-volatile compounds, such as turmeric powder, are commonly used as spices. This research focuses on extracting essential oils from the rhizome to highlight the volatile substances present.Turmerone, Tr-Curcumin, and Curlone are key components in turmeric oils, contributing to their antioxidant and antimicrobial properties. Turmeric offers various health benefits, including anti-inflammatory, antitumor, and antiprotozoal properties, making it a popular ingredient in the cosmetics and pharmaceutical industries[9,10].

Figure 2: *Curcuma longa*

Figure 3: *Azadirachta indica*

Azadirachta indica, also known as Neem, is a plant from the Meliaceae family. It is used in traditional medicine for its therapeutic properties. Neem has antibacterial, antifungal, and antiviral activities. It also has various pharmacological benefits such as being an antioxidant, antimalarial, anti-inflammatory, and anti-diabetic. Neem leaves can be used to treat eczema, acne, diabetes and other conditions. Our study aims to explore the antibacterial and antifungal activities of neem leaves on harmful bacteria [10, 11].

Materials and methodology

Strains used:

Escherichia coli- The bacterium *Escherichia coli*, also known as *E. coli*, is facultatively anaerobic, rod-shaped and gram-negative. Theodor Escherich originally reported this bacterium in 1885. As a typical part of the gut flora, the majority of E. coli strains inadvertently invade the digestive tracts of humans and animals. But certain strains of E. coli have developed into dangerous strains by gaining virulence factors from bacteriophages, transposons, plasmids, and/or pathogenicity islands [11]. A large number of E. coli genomes have been sequenced; these genomes show variations in size and genetic diversity between commensal andpathogenic bacteria, suggesting a wide range of variations within the same bacterial species. They are made up of commensals, or non-pathogenic microorganisms [12].

Bacillus subtilis- Numerous Bacillus strains can be found in the environment, including the human gut, soil, air, and fermented foods. The spore form of Bacillus probiotics can withstand harsh environmental conditions, allowing for long-term life in settings that would normally be fatal for vegetative bacteria. Probiotic Bacillus spores have been shown to germinate, develop, and resporulate throughout the gastrointestinal tract. Numerous strains of Bacillus, such as *B. clausii*, *B. pumilus*, *B. polyfermenticus* and *B. subtilis*, have been the subject of recent investigations. Because Bacillus stains can transfer genes linked to antibiotic resistance, enterotoxins, and biogenic amines (BA), employing them as probiotics has significant drawback [13].Bacillus species have the ability to release compounds that promote plant development and health in addition to forming endospores that are incredibly resilient to adverse environmental conditions. Therefore, the effective use of beneficial microorganisms offers a blueprint for improving stress tolerance and climate change adaptation [14].

Colonization of roots by *Bacillus subtilis* is beneficial to both the bacterium and the host plant.By means of root exudates, plants release about 30% of the fixed carbon they make. After bacteria colonize the roots, plants receive a supply of nutrients in return. The substances and activities produced by the bacteria promote plant growth and protect the hosts from stress. As a means of long-term colonization, Bacillus subtilis creates a thin biofilm on the roots [15].

Saccharomyces cerevisiae-The unicellular fungus *Saccharomyces cerevisiae*, sometimes known as *S. cerevisiae*, is a model organism and an important resource for all facets of fundamental study. However, in contrast to other model organisms like *Escherichia coli* and *Caenorhabditis elegans*, *S. cerevisiae* is also a very desirable species for a wide range of commercial uses [16].

S. cerevisiae is a eukaryote with a nucleus and other organelles that are membrane-bound and is tiny in size. Since yeast cells don't require a complicated growth media, they are comparatively simple to cultivate in a laboratory setting. Furthermore, under ideal laboratory settings, cells divide quickly—every 90 minutes—by budding, a process that results in the bud of a smaller, genetically identical daughter cell from the mother cell during mitosis.For thousands of years, unicellular yeast cells called *Saccharomyces cerevisiae* have been utilized to produce alcohol and baked goods [17].

Chemicals used

Methanol

Collection of parts of medicinal plants

Figure 4: Identification certificate of Neem

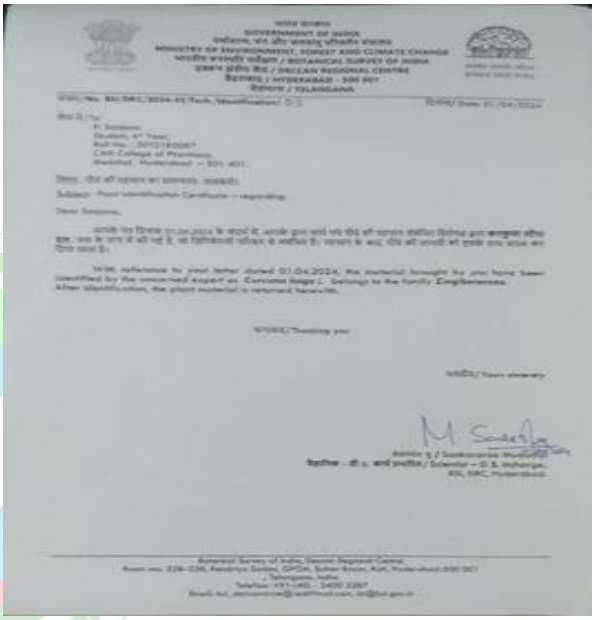
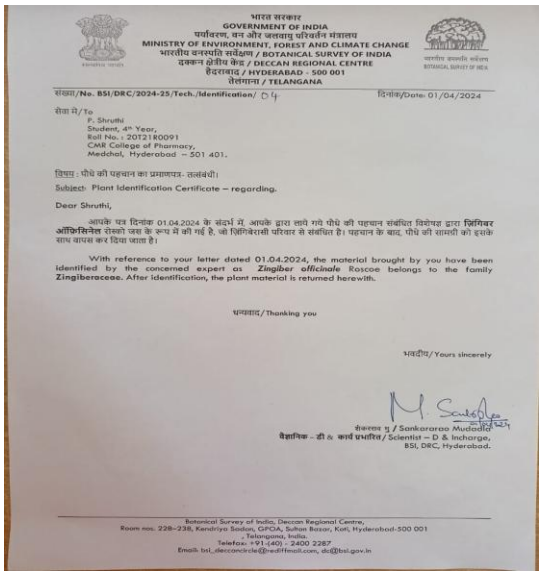


Figure 5: Identification certificate of turmeric

Ginger, neem and turmeric were gathered from different places of Telangana. The neem leaves, ginger, and turmeric rhizomes were then dried in room temperature. Authentication was done for 3 plants at Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana.



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GOVERNMENT OF INDIA
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MINISTRY OF ENVIRONMENT, FOREST AND CLIMATE CHANGE
भारतीय वनस्पति सर्वेक्षण / BOTANICAL SURVEY OF INDIA
दक्कन क्षेत्रीय केंद्र / DECCAN REGIONAL CENTRE
हैदराबाद / HYDERABAD - 500 001
तेलंगाना / TELANGANA

संख्या/No. BSI/DRC/2024-25/Tech./Identification/ 05 दिनांक/Date: 01/04/2024

सेवा में/To,
P. Shruthi
Student, 4th Year,
Roll No. : 20121R0091
CMR College of Pharmacy,
Medchal, Hyderabad – 501 401.

विषय : पौधे की पहचान का प्रमाणपत्र- रलंबंधी।
Subject: Plant Identification Certificate – regarding.

Dear Shruthi,

आपके पत्र दिनांक 01.04.2024 के संदर्भ में, आपके द्वारा लाये गये पौधे की पहचान संबंधित विशेषज्ञ द्वारा **जिंगिबर ऑफिसिनेल** रोस्को जंश के रूप में की गई है, जो जिंगिबेरासी परिवार से संबंधित है। पहचान के बाद, पौधे की सामग्री को इसके साथ वापस कर दिया जाता है।

With reference to your letter dated 01.04.2024, the material brought by you have been identified by the concerned expert as ***Zingiber officinale*** Roscoe belongs to the family **Zingiberaceae.** After identification, the plant material is returned herewith.

धन्यवाद/Thanking you

भवदीय/Yours sincerely

वैज्ञानिक - डी व कार्य प्रभारित / Scientist – D & Incharge,
BSI, DRC, Hyderabad.

Botanical Survey of India, Deccan Regional Centre,
Room nos. 228–238, Kendriya Sadan, GPOA, Sultan Bazar, Koti, Hyderabad-500 001
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Email: bsi_deccancircle@rediffmail.com, dc@bsi.gov.in

Figure 6: Identification certificate of ginger

Extractiontechniques

The active ingredients of the plants were systematically extracted by cold maceration technique. Neem leaves, ginger and turmeric rhizomes were air-dried for a period of 3-4 days. Neem leaves were grounded into a fine powder and dissolved in 100ml of solvent (methanol) for 2 days. Ginger and turmeric rhizomes were peeled, cut into small pieces, dried and soaked in 100ml of methanol for 2 days respectively. The mixture was stirred daily and after 48 hours, the ingredients were filtered using Whatman filter paper. [2,15]

Methanolic Extraction

After 48 hours of cold maceration, the solution was filtered using muslin cloth and the filtrate was concentrated on the water bath to evaporate the methanol[2,15]. Then these extracts were used for determining antibacterial and antifungal activities.

Table 1: Preliminary Phytochemical Screening

Tests	Ginger	Turmeric	Neem
a) Test for Reducing Sugars			
➢ Benedict's Test	+	+	+
➢ Fehling's Test	+	+	+
b) Test for Monosaccharides			
➢ Barfoed's Test	-	-	-
c) Test for Amino acids			
➢ Ninhydrin test	-	-	-
d) Test for proteins			
➢ Biuret test	-	-	-
e)Test for Alkaloids			
➢ Dragendorff's test	+	+	-
f) Test for Tannins			
➢ Ferric chloride test	-	+	+

- \+ means the phytoconstituents are present.
- \- means the phytochemicals are absent.

CULTURE MEDIA PREPARATION

Procedure:

Nutrient agar medium (Semi solid media) used for determining the antibacterial activity

Beef extract -2gm

Sodium chloride- 1gm

Peptone- 2gm

Agar -3gm

Water -200ml

Sabouraud dextrose agar media used for determining the antifungal activity

Both pathogenic and non-pathogenic species of yeasts and fungi can be isolated, cultivated, and maintained on Sabouraud Dextrose Agar (SDA).

Dextrose- 4gm

Peptone- 1gm

Agar- 1.5gm

Distilled water- 100ml

Inoculum preparation

Stock cultures of bacteria and fungi were created in the lab. The stock culture of gram-negative bacteria *Escherichia coli*,gram-positive*Bacillus subtilis*and fungi *Saccharomyces cerevisiae* were prepared. These cultures were stored on nutrient agar media and kept at 4°C. An active culture was made in nutrient broth for the experiment by transferring cells from the stock cultures into a test tube. The same process was repeated for each sample of bacterial and fungal cultures. Agar disc diffusion was carried out for the assay[18].

Method for Disc Diffusion:

The antibacterial and antifungal properties of the provided samples were evaluated using a technique called the disc diffusion method. First, a nutrient agar media was prepared by mixing it with distilled water at a specific concentration for bacteria and sabouraud agar media was prepared for fungi. The media and petriplates were sterilized before pouring the media into the petriplates and allowing it to solidify. After solidification, bores were made in the petriplates and the herbal extract was poured into them to check for antimicrobial activity. The petriplates were then left undisturbed for 15-20 minutes before being incubated in an incubator set at 37°C for 24 hours. The zone of inhibition was observed and the diameter was measured to determine the growth of bacteria and fungi and the activity of that specific concentration of plant extract on the disc [19,20].

Table.2: Comparison of Zone of inhibition of different herbal extracts

Herbal extracts	Zone of inhibition in *E. Coli* (in mm)	Zone of inhibition in *Bacillus subtilis* (in mm)
Ginger	12 mm	11.5 mm
Turmeric	11 mm	11.5 mm
Neem	12 mm	12 mm

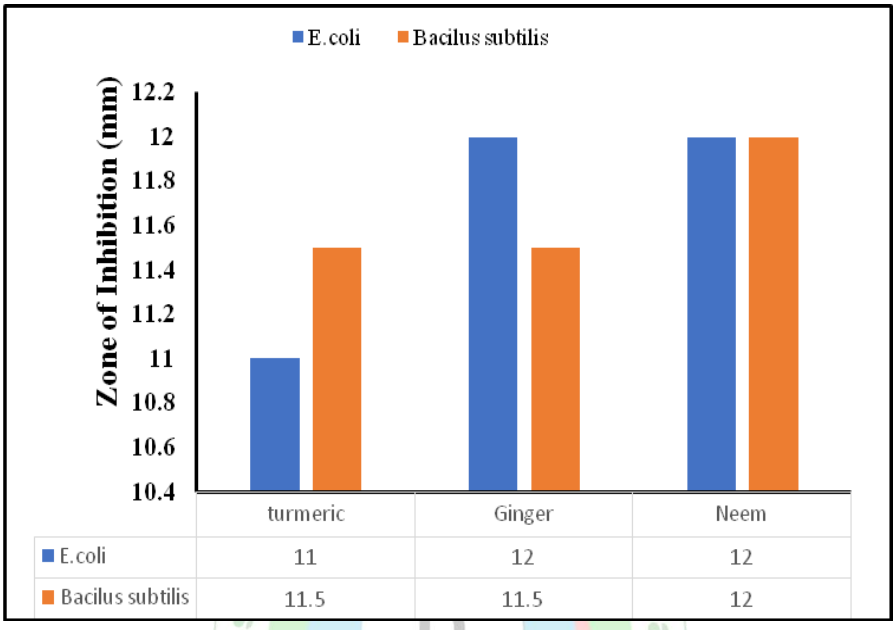

Figure.7: Comparison of zone of inhibition of herbal extracts in *E. Coli* and *Bacillus subtilis*

Table.3: Comparison of zone of inhibition of different herbal extracts in *Saccharomyces*

Herbal extracts	Zone of inhibition in *Saccharomyces cerevisiae* (in mm)
Ginger	8 mm
Turmeric	9 mm
Neem	10.5 mm

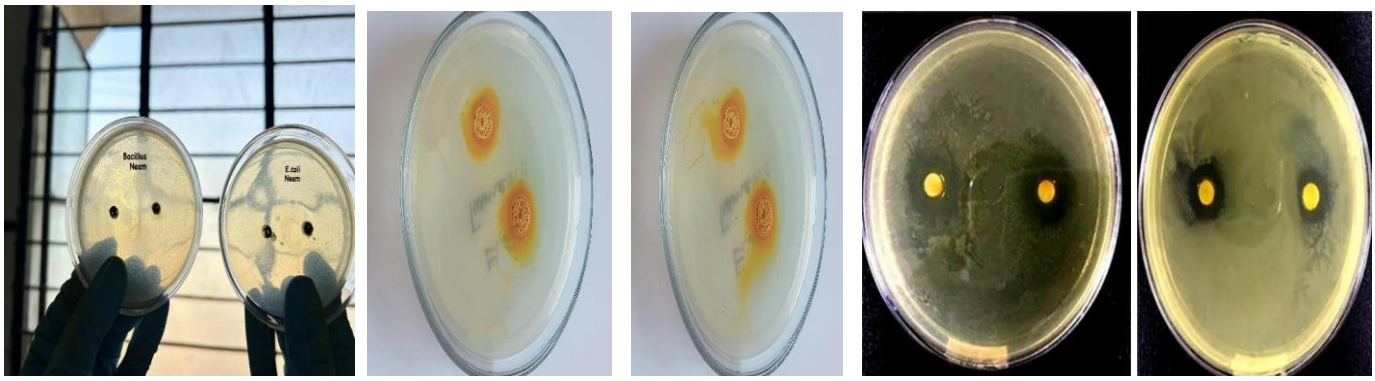

Figure 8: Zone of inhibition of neem, ginger and turmeric in *Saccharomyces cerevisiae* respectively

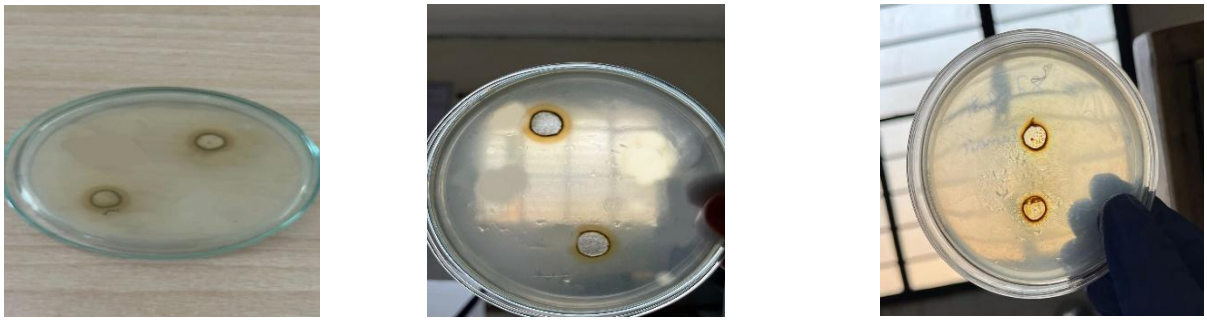

Figure 9: Zone of inhibition of neem, ginger and turmeric in *Saccharomyces cerevisiae*

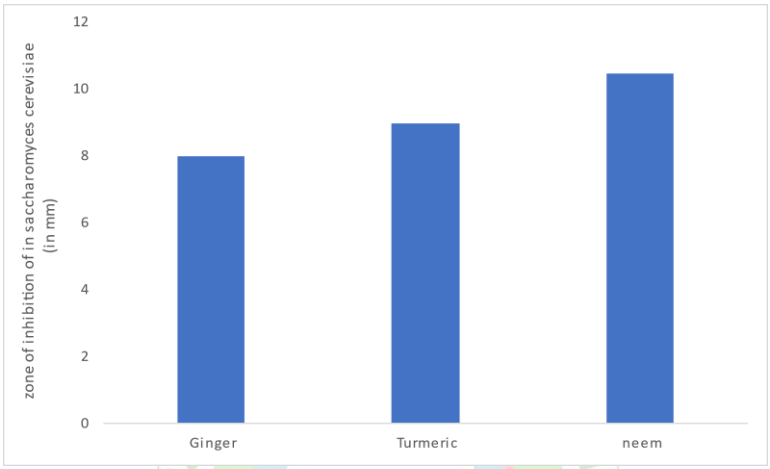

Figure 10: Comparison of zone of inhibition of herbal extracts in Saccharomyces cerevisiae

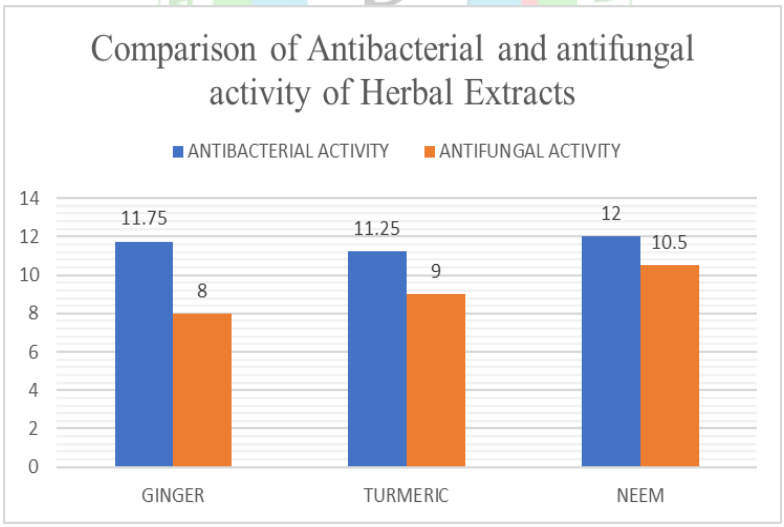

Figure 11: Comparison of antibacterial and antifungal activity of herbal extracts

RESULTS AND DISCUSSION

Plant extracts contain a large number of bioactive compounds. The phytochemical screening is important and thus the screening of compounds exhibiting the antimicrobial activity is necessary. The zones of inhibition against *E. Coli* and *Bacillus subtilis* are visualized on the plates (Table 2). This method is considered to be convenient for obtaining the reliable information on the antimicrobial activities.

The results of antibacterial activity of methanolic extract of ginger, turmeric and neem is given in the table 2. It shows that ginger and neem are equally effective against *E. Coli* and neem is more effective against *Bacillus subtilis* (Fig 4).

The results of antifungal activity of methanolic extract of ginger, turmeric and neem is given in the table 3. It shows that neem is more effective against *Saccharomyces cerevisiae*. This indicates that these herbal extracts have potential antimicrobial activity. Combination of these extracts might show enhanced activity.

When the antibacterial and antifungal properties of the herbal extracts were compared, the antibacterial activity of each extract was higher. Neem exhibited more antifungal efficacy,

while ginger displayed the strongest antibacterial action (Fig.11).

CONCLUSION

From the above study, it can be concluded that all the plant extracts showed promising antibacterial and antifungal activity. The methanolic herbal extracts of ginger, turmeric rhizomes and neem leaves exhibit promising antibacterial properties against E. Coli and Bacillus subtilis and antifungal properties against Saccharomyces cerevisiae. Extracts exhibiting variations in the antimicrobial activity is due to differences in the composition of bioactive compounds extracted depending upon the polarity of the solvent.

Variations in the antibacterial and antifungal activity of the extracts are attributed to the presence of a wide range of active constituents. It is plausible that the combination of these extracts may lead to enhanced activity compared to individual extracts. Therefore, these extracts can be utilized as additives in food and as preservatives to regulate the growth of microorganisms, thereby safeguarding the well-being of both humans and animals. Further research is going on in this area to isolate and characterize the bioactive compounds of neem, turmeric and ginger which may provide a scope of developing more effective drugs for combating infectious diseases.

The use of herbal preparations for food preservation, the treatment of infectious disorders brought on by pathogenic bacteria, and the prevention of microbial deterioration of food products has been suggested by research on herbal extracts. Potential uses for the isolated and

Characterised active chemicals with biological activities of these herbal plants include the development of a novel medication.

CONFLICT OF INTEREST

The authors declared that there is no conflicts of interest regarding the publication of this paper.

ACKNOWLEDGEMENT

We are thankful to the management and the Principal of CMR College of Pharmacy specially the Department of Pharmaceutical Chemistry for providing the facilities such as lab, equipment and chemicals to carry out this research work.

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