IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

A REVIEW ON PHARMACOLOGICAL ACTIVITIES OF AZADIRACHTA INDICA, TRIGONELLUM FOENUM GRAECUM.L

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INTRODUCTION

India boasts a diverse spectrum of climate conditions, topography, and abundant wildlife. It is regarded as the world's richest biodiversity hotspot because it is also home to a large number of plant species.Many African, European, and Asian nations continue to use traditional medicine, which uses isolated chemical compounds from various medicinal plants to treat a range of ailments, even in this day and age of modern medical technology. In addition to ancient medicines, many contemporary medications that are widely available today also contain plant components and are derived from a wide range of flora species. There are less adverse effects .

A significant portion of the population in developed countries is demonstrating interest in herbal remedies, and pharmaceutical companies are focusing much of their attention on developing medical products that can be used to treat a wide range of illnesses.Natural products made from plant ingredients are crucial for creating formulations that are therapeutically active for the majority of acute and chronic illnesses as well as other pharmacological uses. In order to regulate innate protective actions and interactions and effectively manage acute, chronic, and infectious diseases, natural products are refined and advanced.

Inflammation:

Inflammation is a crucial biological response to harmful stimuli, including pathogens, damaged cells, and irritants, involving immune cells, blood vessels, and molecular mediators. Its function is to eliminate cell injury, clear necrotic cells and tissues, and initiate tissue repair, ultimately preventing further damage.8

Basic Cardinal Signs:

- Calor (heat)
- Rubor (redness)
- Tumor (swelling)
- Dolor (pain)
- Function laesa (loss of function)[8]

Types of Inflammation:

- Acute Inflammatory
- Chronic Inflammatory

Acute Inflammatory: Acute inflammation, lasting a few days, occurs immediately after injury and is triggered by cytokines and chemokines. Caused by pathogens, allergens, toxins, burns, and frostbite, it can serve as a defense mechanism against injury. Sub-acute inflammation, lasting 2-6 weeks, is considered more severe.7

Chronic Inflammatory:

Chronic inflammation, characterized by macrophages, lymphocytes, and plasma cells, can lead to diseases like diabetes, cardiovascular disease, allergies, and COPD. Factors such as obesity, smoking, stress, and insufficient diet can promote chronic inflammation, affecting the body's ability to fight off infections and maintain health.9

Inflammatory response mechanisms:

[9] The inflammatory response involves activating signaling pathways to regulate inflammatory mediator levels in tissue cells and inflammatory cells from the blood. It is a common cause of chronic diseases like cardiovascular and bowel diseases, diabetes, arthritis, and cancer. The mechanism of inflammatory response varies depending on the initial stimulus and location.

- Cell surface pattern receptors recognize detrimental stimuli;
- Inflammatory pathway is activated;
- Inflammatory markers are released;
- Inflammatory cells are recruited[11].

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDs):

NSAID are the medicines are used to treat pain and redness, swelling, and heat (inflammation) from medical conditions such as: different types of arthritis menstrualcramps and other types of short-terms pain[12].

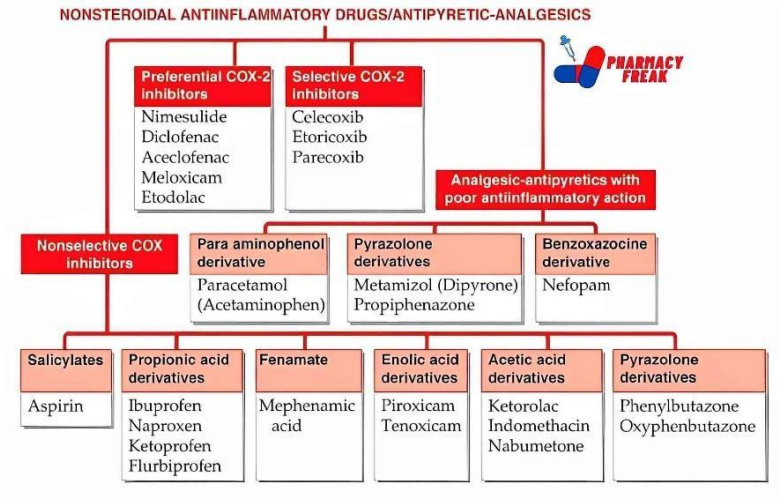

Fig no 3: Classification of NSAIDS.

MECHANISM OF ACTION OF NSAIDS:

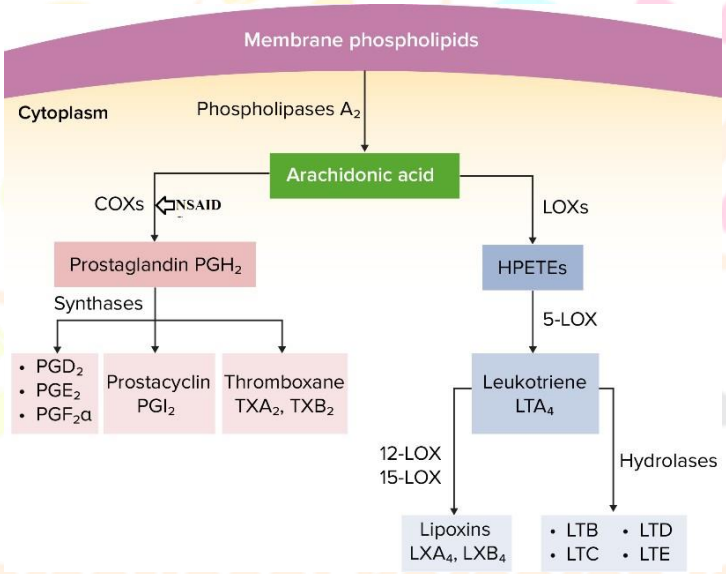

Fig no 4: Mechanism of Action.

Plant profile

AZADIRACHTA INDICA

Synonym: neemDescription:

Azadirachta indica, commonly known as neem, margosa, nimtree or Indian lilac, is a tree in the mahogany family Meliaceae. It is one of two species in the genus Azadirachta. It is native to the Indian subcontinent and to parts of Southeast Asia, but is naturalized and grown aroundthe world in tropical and subtropical areas.

Chemical constituents:

- Alkaloids (ervine, methyl erevine,ervolanine, aervive, methyl aervine, aervoside,ervolanine&aervolanine).
- Limonoid(limolin,nomilin,nomilinic acid)
- glycerides, polyphenols, nimbolide, triterpenes
- Flavonoids (kaempferol, quercetin, persinol ,).
- benzoic acid, β-sitosteryl acetate & tannic acid.

Uses:

Diuretic, immunomodulatory, anti-inflammatory,antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant,Antimutagenic,anticarcinogenic, Hypoglycemic, Anti- diabetic, Anti-urolithiasis, Anti-asthmatic, Anti-fertility, Hypolipidemic.

TRIGONELLUM FOENUM GRAECUM.L

Synonym:fenugreek

Descrption: An annual herb, fenugreek (also known as methi) is mostly grown for its seeds and leaves , which can be dried or fresh.To enhance the taste and nutritional content of meals, seeds are used as seasonings and condiments.Originating from South Eastern Europe and South Western Asia, fenugreek is a significant seed spice. India is in the lead. More than 80% of the nation's fenugreek is produced in Rajasthan alone; other states that are experiencing growth include Gujarat, Madhya Pradesh, Chattisgarh, and Uttar Pradesh.

Chemical constituents:

- Alkaloids: Trimethylamine, Neurin, Trigonelline, Choline, Gentianine, Carpaine,Betain
- Amino acids: Isoleucine, 4-Hydroxyisoleucine, Histidine, Leucine, lysine, l-tryptophan, Arginine
- Saponins :Graecunins, fenugrin B, fenugreekine, trigofoenosides A–G
- Flavonoid:s quercetin,rutin,vitexin,isovitexin
- Lipids :triacylglycerols,phosphatidylcholine

Uses:

antidiabetic, antioxidative,hypocholesterolemic, antineoplastic,anti-inflammatory, antiulcerogenic, antipyretic, immunomodulatory and antitumor

PHARMACOLOGICAL ACTIVITIESNEEM

ANTIMICROBIAL ACTIVITY

In vitro techniques like broth dilution, disc or agar diffusion, and agar overlay assays are frequently used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of each treatment in order to test the antimicrobial activities of neem oil extracts and phytochemicals. To better replicate human infection and disease testing, a few in vivo models have been put into place. These models involve injecting drugs intraperitoneally or intravenously, or giving neem oil-related medications orally or gastrically to mice, rats, guinea pigs, and rabbits. These published studies on animals show that the plant component and solvent used to make the extract, along with the treatment route and species used in the model, all have a significant impact on the acute toxicity level of neem.

ANTIBACTERIAL ACTIVITY

A large portion of recent research on the antimicrobial potential of neem has concentrated on the plant's antibacterial qualities due to the growing rates of antibiotic resistance for bacterial pathogens and the consequent need for novel therapeutics. The successful applications of neem in the food industry and the traditional use of neem products for dental hygiene lend support to this area of research. The capacity of pathogenic bacterial species to form biofilms has sparked interest in elucidating how these communities contribute to enhanced tolerance to antibacterial substances, in addition to standard antibiotic resistance. Despite the widespread recognition of the significance of biofilm-associated infections in human disease, few innovative methods for successfully eradicating biofilms have been developed to date.

ANTIDIABETIC ACTIVTY

Streptozotocin induced models of IDDM (65 mg/kg, iv) as well as NIDDM model (90 mg/kg, i.p in neonates) were given neem leaf extract (NLE, 1 g/kg, po) for 6 weeks and their anti- diabetic activity was assessed.

Glucose Tolerance Test: Animals were overnight fasted. Fasting blood sugar levels were detected by taking blood samples. Then the test drugs were administered orally 60 minutes prior to administration of glucose to the 5 groups of animals each containing 6 animals. After 60 minutes glucose (in the dose of 1.25 grams/kg) was administered orally to each rat. Blood samples were drawn every half hourly up to 4 hours and blood sugar levels were detected by glucometer. The blood was taken by chopping tail of rat.

Alloxan Induced Diabetes: Overnight fasted animals were given 100 mg/kg alloxan monohydrate.After 48 hours blood sugar levels were estimated by glucometer. Then the animals were divided into 5 groups. In each group 6 rats were kept. All rats received drugs orally for 15 days. In pilot study NRE in single daily dose reduced blood sugar levels but they were not significant. So we gave NRE twice daily.The blood was taken by chopping tail of rat.Blood sugar level was measured on 48 hours, 5,10 and 15 days by using glucometer.

Alloxan monohydrate: More than 300 grams and less than 250 grams of rat weight. female rats who are pregnant and those who have given birth once.Following an overnight fast, fasting blood glucose levels were measured in every rat. Samples of blood were obtained from the vein of a rat tail, after the veins were highlighted with xylene. A glucometer was used to estimate blood glucose. Each group underwent an oral glucose tolerance test following 28 days of treatment.

ANTIOXIDANT ACTIVITY

Cupric ion reducing (CUPRAC) method:

To the reaction mixture containing CuCl2 (1 ml, 10 mM), neocuproine (1 ml, 7.5 mM), and NH4Ac buffer (1 ml, 1 M, pH 7.0), a test solution (0.5 ml) was added. Likewise, a blank (0.5 ml sample solution and 3 ml reaction mixture) devoid of CuCl2 were made for every sample. After 30 minutes of incubation at room temperature, the absorbance at 450 nm was measured. The findings were presented in terms of milligrams (mg TEs) of Trolox equivalents per sample amount Citation 28.

Ferric reducing antioxidant power (FRAP) method:

Acetate buffer (0.3 M, pH 3.6), 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl, ferric chloride (20 mM), and sample solution (0.1 ml) were added to FRAP reagent (2 ml) in a ratio of 10:1:1 (v/v/v). The absorbance was then measured at 593 nm following a 30- minute room temperature incubation period. The findings were presented in terms of milligrams (mg TEs) of Trolox equivalents per sample amount Citation.

Metal chelating activity on ferrous ions:

FeCl2 solution (0.05 ml, 2 mM) was mixed with test solution (2 ml). The addition of 5 mM ferrozine (0.2 ml) started the process. In a similar manner, a blank was made for each sample, which included water (0.2 ml), FeCl2 solution (0.05 ml, 2 mM), and sample solution (2 ml). The sample and blank absorbance was then measured at 562 nm following a 10-minute incubation period at room temperature. The results were given in milligrams (EDTAEs) Citation 30 of EDTA equivalents per sample quantity.

ANTIFUNGAL ACTIVITY

HPLC analyses and chromatographic purification of nimonol :

Using ethyl acetate made from neem leaves, the various components of the mother organic extract were analyzed using method (8). The following components of the HPLC system (Perkin-Elmer, Norwalk, CT, USA) were used to fractionate the organic extract: Using Acherey-Nagel 100 C-18 columns (20 mm x 25 cm, 215 nm) and an LC 90 UV spectrophotometric detector, a 410 LC pump is outfitted with an LCI 100 integrator at 230 nm. Methanol (Carlo Erba, Milan, Italy) and ultra pure water (purified using a Milli-Q system; Millipore, Bedford, MA, USA) were included in the mobile phase. The two-hour chromatographic run involved 20 µl of samples, each comprising 3 mg of EtoAc extract diluted in 1 ml of methanol at a flow rate.

In vitro assessment the antifungal activity of the neem extract:

Glass Petri dishes with a diameter of 9 cm were filled with the NE after it had been dissolved in sterilized PDA at 45°C (resulting in a final concentration of 100 mg NE per ml PDA) (20 ml each dish). From one-week-old cultures on PDA plates, a single 6 mm disc of the fungal species was cut, and each disc was then placed in the center of the dish. A 50 l conidial suspension of P. expansum (10 6 conidia per ml) was put into the 6 mm disc that was removed from the PDA in the center of each dish (one hole per dish) for P. expansum analysis. The dish was then incubated. Growth of the pathogens was significantly ($P < 0.05$) inhibited by the NE. As shown in Table 1, the index of growth inhibition respectively was

65.7% for the M. fructicola, 34.1% for the P. expansum, 66.9% for the T. roseum and 30.7% for the A. alternate, respectively, after incubated at 27°C for 72 h .

ANTIANALGESIC ACTIVITY

The analgesic property of albino rats was investigated by randomly assigning them to several groups. Ten rats (n=10) were chosen from each group. In this investigation, normal saline was employed as the vehicle and morphine sulphate was used as the reference standard medication. NLE was the test medication, and it was dissolved in distilled water. Both the test medication NLE and the standard drug morphine were administered intraperitoneally while adhering to all aseptic procedures. Every intraperitoneal injection was administered at a consistent volume of 0.5 milliliters. The tail flick response to thermal stimulation experimental pain model was used to evaluate the analgesic effects of the two medications.
Using an analgesiometer, the heat stimulation was administered. A nichrome wire is located at the top of the closed analgesiometer.

ANTIINFLAMMATORY ACTIVITY

An acute inflammation model was used to assess neem leaf extract's anti-inflammatory properties. According to a previously published procedure, rats' right hind paw plantar aponeurosis was injected with 0.1 ml of a 1% carrageenin suspension in normal saline to induce acute inflammation in the form of hind paw edema. For every rat in the control group,
0.1 ml of normal saline was injected concurrently behind the plantar aponeurosis of the left hindpaw. On both hind limbs, right past the tibio-tarsal joint, a "A" mark was formed. The water displacement method was used to estimate the volume of paw edema, and the displaced water was gathered in a microburette. For this, a glass tube with a side outlet was employed. were taken as significant. The percentage of inhibition of paw edema was calculated by the formula used by previously described method

Vc-Vt X 100 Vc

Where: Vc = Mean volume of paw edema in the control group of animals. Vt = Mean volume of paw edema in the drug treated group of animals.

ANTI PYRETIC ACTIVITY

The albino rats used in this investigation were split into groups of six at random. The rats were acclimated to room temperature and humidity levels for seven days in separate cages in the laboratory prior to the experiment. The animals' rectal temperatures were taken every day at 9:00 am and 9:00 pm to determine if there is a daily fluctuation in the temperature. For seven days, this process was carried out. The animals had unlimited access to food and drink. The study comprised the animals whose rectal temperatures varied by less than 10c or remained constant. A clinical thermometer was used to record rectal temperatures.

Using the brewer's yeast, the antipyretic investigation was conducted in accordance with a previously published approach .

FENUGREEK

ANTIINFLAMMATORY ACTIVITY

Carrageenan-Induced Paw Edema:The method used was that suggested by Singh and Ghosh, which employed a plethysmometer.Vehicle,Volume of the paw was measured at 0 h and after 3 h with a plethysmometer. Where VT is the change in the paw volume in the treatment group and VC is the change in the paw volume in the control group.Carrageenan, an irritant, inflammogen, and antiphlogistic substance, is commonly employed in the research of acute and subacute phases of inflammation in rodents. The sub-plantar area of the left hind paw is injected with a freshly made 1% volume suspension of carrageenan in normal saline (typically 0.1 ml in rats and 0.025–0.05 ml in mice). The animals in the control group receive injections just from the vehicle. Usually, the test medication was taken orally within an hour of the carrageenan challenge, based on bodyweight. The tested rat had a mark produced at the ankle joint. Following the carrageenan challenge, paw volume up to the ankle joint was measured in the drug-treated and untreated groups at 0 min, 30 min, 60 min, and 120 min .

ANTIDIABETIC ACTIVITY

The patients were split into two groups: the experimental group received fenugreek seed powder in addition to their regular treatment, while the control group received only their regular anti-diabetic medication. Patients were instructed to arrive after fasting during the night, and early in the morning, blood samples were taken. The control group was instructed to maintain their regular diabetic care (medication or diet control with exercise) after a blood sample was obtained on day 1 as a baseline record. On days 21 and 42, two further blood samples were collected in order to examine the serum lipid levels. Day 1 of the experimental group's treatment involved taking a blood sample as a baseline record and advising them to continue with their regular diabetes regimen (i.e., medication or food control with exercise).

ANTI OXIDANT ACTIVITY

Ferric Reducing Antioxidant Power (FRAP):The concept of measuring antioxidant activity using ferric reducing antioxidant power, or FRAP, was first put forth by [11]. First, fresh 300 mM acetate buffer FRAP reagent was made in the manner described below: pH 3.6 (10 mM 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl; 20 mM FeCl3·6H2O in the ratio of 10:1:1 to provide the working reagent; and 3.1 g sodium acetate trihydrate plus 16 mL glacial acid made up to 1:1 with distilled water). Additionally, after 30 minutes, a spectrophotometer was used to measure the absorbances at a wavelength of 595 nm after adding around 1 mL of FRAP reagent to 100 µL of fenugreek extracts. Trolox's calibration curve was developed to estimate the sample activity capacity.

DPPH Radical Scavenging Activity: A 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system was used to measure the antioxidant activity in accordance with the methodology of 40 mg of DPPH were dissolved in 100 mL of methanol to create the stock solution, which was then kept at -20 °C until needed again. Using a spectrophotometer (Epoch, Biotek, USA), about 350 mL of stock solution and 350 mL of methanol were combined to get an absorbance of 0.70±0.01 unit at 516 nm wavelength. For the purpose of scavenging reaction, 100 µL extracts of Trigonella foenum-graecum seeds and 1 mL of methanolic DPPH solution were produced and stored overnight in the dark. The following formula was used to calculate the percentage of DPPH scavenging activity:

DPPH scavenging activity (%) = [(A blank –A sample) / A blank] × 100, where A is the absorbance.

HYPERLIPIDEMIC ACTIVITY

To develop hyperlipidemia, all 20 rabbits were randomly split into two groups of ten each, and each group was given 2 ml of edible oil (coconut oil) and 0.5 g/kg body weight/day of pure cholesterol (Sigma, USA) orally through an oral feeding tube. This was done for four weeks. After causing hyperlipidemia, the cholesterol feed was stopped. For four weeks, Group-I (n=10, hyperlipidemic rabbits) received 2 ml of aqueous emulsified whole fenugreek seed powder (500 mg/kg body weight / day) orally via an oral feeding tube. The rabbits were kept on a regular standard diet. For four weeks, Group-II (n=10, hyperlipidemic rabbits) received a typical normal diet plus 2 milliliters of an aqueous atorvastatin emulsion (0.5 mg / kg body weight / day). All the parameters of lipid profile i.e. serum total cholesterol, LDL-cholesterol, triacylglyceride and HDL-cholesterol were done.HYPOGLYCEMIC ACTIVITY

Wistar rats were employed to assess the anti-diabetic effects, and locally sourced, freshly harvested seeds were procured. By grinding the seeds, a fine powder was produced. Soxhlet extraction, also known as Soxhelation (100 g), is a continuous hot percolation procedure that was used for three to four days to produce extracts of powdered seeds with 90% ethanol.[18] Every control group was given a single milliliter of normal saline as a vehicle.150 mg/kg of alloxan monohydrate is used to cause diabetes mellitus. A single intraperitoneal injection of alloxan (150 mg/kg body weight) dissolved in normal saline administered to the Wistar rats overnight fasting resulted in the development of diabetes mellitus. After alloxan was administered to rats for 72 hours, the rats' diabetes was evaluated, and blood glucose levels (BGL) were measured after 0 days, 7 days, 14 days, and 21 days. Rats with BGL levels higher than 250 mg/dl were chosen for the experimental investigations.T. foenum-graecum extract was fed to the diabetic rats for 21 days at 200 mg and 400 mg/kg, respectively. Alloxan was given to every rat, and the untreated diabetic control group is represented by this group. Group I consisted of six animals, while Group II consisted of gliclazide.

ANALGESIC ACTIVITY

Hot plate technique :Mice of either sex and weighing 20–25 g were split into three groups for this procedure. For four days, these mice received the standard saline (10ml/kg) intraperitoneally, T. foenumgraecum sprouts (200mg/kg) intraperitoneally, and diclofenac sodium (10mg/kg) intraperitoneally. The animals were put through a hot plate test to determine their level of analgesia four days later, thirty minutes after the last dose was administered. The hot plate's temperature was adjusted to 55 °C. For every group, the latency time on a hot plate without licking or leaping was computed. For the animals, a 30-second cutoff duration was established in order to avoid tissue injury.

The writhing method generated by acetic acid :Mice of either sex and weighing 20–25 g were split into three groups for this procedure. For four days, the following treatments were given to the animals beforehand: 10 mg/kg of normal saline, 200 mg/kg of T. foenum-International Journal of Research in Pharmacy and Pharmaceutical Sciences 69 graecum sprouts, and 10 mg/kg of diclofenac sodium. 30 minutes following the last dose, on day four, the animals received intraperitoneal (i.p.) treatment with 1% acetic acid. The number of abdominalconstrictions was counted for ten minutes following the five-minute acetic acid injection.

The following formula was used to calculate the percentage inhibition of pain.A-B/A× 100

A= number of writhes in the group under controlB= the amount that the tested group writhed.

ANTI PYRETIC ACTIVITY

Mice of either sex and weighing 20–25 g were split into three groups for this procedure. Each animal's normal body temperature was determined via the rectal route using a digital thermometer prior to the administration of the dose. After that, the rats received two days' worth of normal saline (10ml/kg) i.p., 200 mg/kg of Trigonella foenum-graecum sprouts, and
10 mg/kg of diclofenac sodium intraperitoneally. After two days, 20% Brewer's yeast suspension (10 ml/kg) was administered subcutaneously to cause pyrexia. Twenty-four hours following the yeast injection, a rectal digital thermometer was used to take the patient's body temperature once more. The following formula was used to compute the percent reduction in pyrexia:

Reduction percentage = A-B/A× 100

Before treatment: A= temperature; after treatment: B= temperatureANTIBACTERIAL ACTIVITY

The paper disc diffusion method was used to assess T. foenum-graecum sprouts' antibacterial activity. Twenty milliliters of blood agar, or sheep blood, was used to make agar plates, which were then placed onto sterile petri dishes. Using a sterile cotton swab, agar plates were infected with bacterial stock suspension and incubated for 15 minutes at 37°C. The test bacterial plate was covered with sterilized 6-mm-diameter filter paper discs and a disc containing the positive control medication, amoxicillin (10 mcg), after 15 minutes. Using a micropipette, dilutions of T. foenum-graecum sprouts (500 mg/ml, 250 mg/ml, 125 mg/ml, and 75 mg/ml) were deposited on the surface of a 75 mcl filter paper disc. The plates were then incubated for 48 hours at 37 °C.

ANTIFUNGAL ACTIVITY

The paper disc diffusion method was used to assess T. foenum-graecum sprouts' antifungal activity. Twenty milliliters of melted agar was used to create agar plates, which were then placed into sterile petri dishes. Using a sterile cotton swab, agar plates were infected with fungal stock suspension and incubated for fifteen minutes. The test fungus plate was covered with sterile 6 mm diameter filter paper discs and a disc containing 10 mcg of the positive control medication Clotrimazole after 15 minutes. Using a micropipette, dilutions of T. foenumgraecum sprouts (500 mg/ml, 250 mg/ml, 125 mg/ml, and 75 mg/ml) were applied to the surface of a 75 mcl filter paper disc. Following a 72-hour incubation period at 37 °C, the inhibition zone was determined on these plates.