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Research Article

IN VITRO ACTIVITY OF MEDICINAL PLANTS AGAINST SOME BACTERIAL AND FUNGAL ISOLATES

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ABSTRACT

Objective: The main aim of this study was to evaluate the antifungal and antibacterial activity of four medicinal plants against some bacterial and fungal isolates.

Methods: Leaves were powdered after drying at 37°C for 3-10 days. Exposure to sunlight was avoided to prevent the loss of active compounds. The completely shade dried material was coarsely powdered and put in soxhlet apparatus for successive extraction with different solvents like petroleum ether, ethyl acetate and methanol. Paper disks containing different solvent extracts of medicinal plants were used to evaluate the *in vitro* antibacterial and antifungal activity by measuring the diameter of inhibition zone around the disks.

Results: Four fungal and three bacterial species were isolated and identified from the soil sample collected from different places of Jabalpur (M.P.). *Aspergillus niger* was most frequent (60%) followed by *Aspergillus flavus* (20%), *Penicillium notatum* (10%), *Fusarium oxysporum* (10%). The three bacterial species viz., *Escherichia coli*, *Bacillus subtilis* and *Entrococcus faecalis* were isolated and identified. Results obtained during assay with plant extracts from *Azadirachta indica*, *Ocimum gratissimum*, leaves showed their inhibitory effect against fungal and bacterial isolates.

Conclusion: Leaves extracts of *Ocimum gratissimum* showed potential inhibitory activity against bacterial and fungal isolates than the other tested ethno-medicinal plants.

Keywords: Isolates, Medicinal plants, Activity.

INTRODUCTION

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. The wide range of medicinal plant parts is used for extract as raw drugs, and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs.

Nature has provided a complete store house remedies to cure all ailments of mankind. The natural or herbal remedies are still the backbone of medicines; phytotherapy is a medicinal practice based on the use of herbal plants and their extracts. These herbs or plants and their active ingredients are used in traditional herbal remedies. The easy availability, low cost and negligible side effects, natural products, are popular in the now the days in the world [2].

India has been known to be a rich repository of medicinal plants since ancient time the agro climate condition prevailing in India provide herbs, which provide the raw material for pharmaceutical food. In developing countries and particularly in India low-income people such as farmers, people of small isolated village and native communities use folk medicines for the treatment of common infection [3].

Madhya Pradesh has treasure of biodiversity in medicinal and aromatic herb. Due to wide variety more than 1100 medicinal plants are used in folk and traditional medicine, which are found in the forest flora of Sapura, tribal's of Sidhi, Shadol, Chitrakoot, Dindori, Amarkantak, Mandala, Seoni, Chindawara, Betul, Sagar, Damoh, Dhar and Kharogan are still using the medicinal herbs for their own treatment and are supplying these to the traders.

All the herbs produced are wildering variety of phytochemicals like primary metabolites (carbohydrates, fats, proteins) and secondary metabolites (alkaloids, flavonoids, steroids, saponins, polyphenols, etc.) for their normal metabolic activities [4]. These secondary metabolites showed various biological activities and act in plant defense mechanisms [5].

Medicinal plants are important substances for the study of their traditional uses through the verification of pharmacological effects and can be natural composite sources that act as new anti-infectious agents. Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care due to better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world.

Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side-effects that are often associated with synthetic antimicrobials. All medicinal, plant contains certain active constituent, it responsible to some pharmacological activity. Medicinal plants serve as the major source of medicines for the treatment of various ailments. According to the World Health Organization, over 80% of the world's population, especially in the developing world relies on medicinal plants as sources of medicines for their primary healthcare [6].

Now a day's multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotic are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reaction. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance there is a constant need for new and

effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants.

There are several reports on the antimicrobial activity of different herbal extracts in different regions of the world because of the side-effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species seed in herbal medicine. Plant based antimicrobials represent a vast untapped source of medicines, and further exploration of plant antimicrobials need to occur.

Traditional system of medicine that depends mainly on medicinal plants is rich in ethnomedical knowledge of the uses of medicinal plants in the treatment of infectious conditions [7]. These medicinal plants employed in traditional medicine represent potential sources of cheap and effective standardized herbal medicines (phytomedicine) and leads to the discovery of novel molecules for the development of new chemotherapeutic agents [8]. Several infectious diseases, including malaria, diarrhea, dysentery, gonorrhea, and fungal infections have been successfully managed in traditional medical practice employing medicinal plants [9].

Some medicinal plants are more efficient to treat infectious diseases then synthetic antibiotic. It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases [10]. Antimicrobial, antioxidant and anti-inflammatory properties of some Indian plants.

Classification

Kingdom - Plantae
Division - Magnoliophyta
Order - Sapindales
Family - Meliaceae
Genus - *Azadirachta*
Species - *Azadirachta indica* (Neem)

Neem (*A. indica*) in the mahogany family, Meliaceae is evergreen tree found in most tropical countries. It is one of the two species in the genus *Azadirachta*, native to India and Burma, growing in tropical and semi-tropical regions. It is a fast growing tree, average height 15-20 m, but rarely to 35-40 m. For thousands of years, the beneficial properties of neem have been recognized in the Indian tradition. The bark powder contains sugar, proteins, amino acids and oil [11]. Polysaccharides such as arabinogalactans and fucogalactoglucoarabinanes have also been isolated from neem bark. Flavonoids, flavones glycosides, dihydrochalcones, tannins and others are also important constituents of bark, leaves, fruits and flowers of neem.

Medicinal uses

Neem tree as "panacea of all disease" However in India it is famous with many other names like "Divine Tree," "Heal All," "Nature's Drugstore," and "Village Dispensary." Traditional and ayurvedic uses of neem include the treatment of fever, leprosy, malaria and tuberculosis. Various folk remedies use as an anthelmintic, antifeedant, antiseptic, diuretic, emmenagogue, contraceptive, febrifuge, parasiticide, pediculicide and insecticide [11]. The bark extract is also used as tonic, astringent and useful in relieving fever, thirst, nausea, vomiting and skin diseases. Traditional uses of twigs for brushing teeth as effective forms of dental care. Neem oil is useful for skin care such as acne, and keeping skin elasticity. Traditionally, patients suffering from chicken pox sleep on the leaves in India owing to its medicinal value. In Ayurvedic, Unani and folklore traditional medicine, different parts of neem were preferred in the treatment of a wide range of afflictions. The extract or oil of neem is effective against inflammatory, analgesic, antipyretic activities, and immune modulatory activities. It also showed an immune-stimulant activity, anti-diabetic, antiulcer effect, *in-vitro* spermicidal.

Classification

Kingdom - Plantae
Division - Magnoliophyta
Order - Euphorbiales
Family - Euphorbiaceae
Genus - Jatropha
Species - *Jatropha curcas* (Ratanjote)

J. curcas (Euphorbiaceae) is a biodiesel plant grown in various parts of India and other tropical countries *J. curcas L.* is a flowering plant, and is thus closely related to other important cultivated plants like rubber tree and castor. The plant, *J. curcas* is a small tree or large shrub, which can reach a height of up to 5 m [12]. *J. curcas* is a multipurpose plant that has a long history of cultivation in tropical America, Africa, and Asia with many attributes, mainly as a potential source of bio-fuel because its seed kernels. *J. curcas* variously known as physic nut, purging nut or pignut [13,14] and "Lapalapa" in Yoruba Language [15].

Medicinal uses

It is used in folklore remedies for treatment of various ailments such as skin infections, gonorrhea, jaundice, and fever [16]. Preparations of all parts of this in the form of decoction are used in traditional medicine and for veterinary purposes.

Classification

Kingdom - Plantae
Division - Magnoliophyta
Order - Solanales
Family - Cuscataceae
Genus - Cuscuta
Species - *reflexa Roxb Cuscuta reflexa* (Amarbel)

C. reflexa Roxb is a rootless, leafless perennial parasitic twining herb of convolvulaceae family known as akashvalli or dodder. It grows on thorny or other shrubs, sometimes completely covering the bushes and trees [17]. It is common throughout India, abundant in Bengal plains. It has no root under the ground, but only grows as a parasitic twinner on other plants, and hence called akaswel. It spread from one host to another, and on each victim, they twine and cling tightly with special branching organs called haustorium. Haustorium penetrates the host and connect to the host xylem as well as to the host phloem and absorb from it both elaborated foodstuffs such as sugar and amino acid. It lives its entire life without attachment to the ground and grows with the help of seeds that are minute produce large quantities.

Medicinal uses

Whole plant infusion is used as a wash for sores. Stem useful in bilious disorders. Fruit used in fever and cough. Seed cold infusion is given as a depurative and carminative in pains and stomach-ache, internally useful in appetizer, digestive, liver stimulant, anthelmintic and reduces intestinal motility. Externally useful in inflammation, pain, hair disorder, conjunctivitis and also used against itch and other skin diseases.

Classification

Kingdom - Plantae
Division - Magnoliophyta
Order - Lamiales
Family - Lameacea
Genus - Ocimum
Species - *Ocimum gratissimum* (Tulsi)

The plant of genus *Ocmium* L.(lemicea*)* are native to throughout the old word tropic and widespread as a cultivated plant an escapade weed and are recognized for its therapeutic potentials *Ocimum sactum* L. (tulsi), *O. gratissimum* L. (ram tulsi), *Ocimum basilicum* L.(ban tulsi).

The genus *Ocimum* comes under Lamiaceae family and is found in many part of the world like tropical and sub-tropical regions of Asia, Africa and Central and South America *O. gratissimum* is a grassy annual plant originated from Iran, Afghanistan and India. It is a source of essential oils and aroma compounds, a culinary herb and an attractive, fragrant ornamental plant. Tulsi is considered as a sacred plant, and its various medicinal properties have been mentioned in ancient medicinal text, Ayurveda. Different parts of this plant are used for treatment of various ailments. *Ocimum* is believed to decrease lipid peroxidation and increase the activity of superoxide dismutase [18]. The constituents of *Ocimum* species have antibacterial, antifungal, antioxidant, and radioprotective activity [19]. Studies show that many *Ocimum* species are useful for the treatment of disorders in central nervous system and also as antidepressant [20].

Medicinal uses

Some of the phytochemicals of medicinal importance present in *O. gratissimum* have already been identified [21]. The ability of this plant to be used in traditional medicine in the treatment of headaches, cough, diarrhea, constipation, warts, kidney malfunctions, nasal polyps and ulcers have also been reported [22]. Further, its action as insecticide, nematicide, fungicide and antimicrobial compound also has been reported.

METHODS

Collection of plant material

Fresh leaves of four different plants *A. indica, J. curcas, C. reflexa* and *O. gratissimum* free from disease were collected from Seoni (Madhya Pradesh) in the month of January-March 2014. The plants were identified at Department of Post-graduate Studies and Research in Biological Sciences, Rani Durgavati University Jabalpur (Madhya Pradesh). The leaves were washed thoroughly 2-3 times with running water and once with sterile distilled water, then air-dried on sterile blotter under shade.

Solvent extraction

Thoroughly washed dried leaves of four plants of *A. indica, J. curcas, C. reflexa* and *O. gratissimum* were dried in the shade for 7-10 days and then powdered with the help of waring blender. 100 g of shade-dried powder was filled in the thimble and extracted successively with petroleum ether, ethyl acetate and methanol, solvents in soxhlet extractor for 48 hrs. The solvent extracts were concentrated under reduced pressure and preserved at 5°C in airtight bottle until further use.

Test microorganism

The test microorganisms (bacterial strain) *Enterococus faecalis, Escherichia coli* and *Bacillus subtilis* was isolated from soil. The test fungi *Aspergillus flavus*, *Aspergillus niger, Penicillium notatum* and *Fusarium oxysporium* were isolated from poultry form and other dumping sites. The bacteria obtained were first sub-cultured in a nutrient broth and incubated at 37°C for 18 hrs whereas the fungal isolates were sub-cultured on a Sabouraud Dextrose Agar (SDA) for 72 hrs at 28±1°C.

Isolation of fungal species from the soil sample

Soil samples were collected from the superficial layer at a depth of 3-6 cm. of four poultry farms and six hair dumping/garbage sites from different areas of Jabalpur. The soil samples were placed in sterile polythene bags, brought to the laboratory and stored overnight at 4°C. Approximately, 18-20 g of soil from each sample were placed in 90 mm sterile petri plates in five replicates. Short (1-2 cm length)

sterilized defatted human hair fragments were scattered on the surface of the soil for baiting. The plates were moistened with an antibiotic solution containing cycloheximide (0.5 mg/ml) and chloramphenicol (0.05 mg/ml). The plates were incubated at room temperature (28±1°C) for a period of 4-6 weeks and remoistened with sterile deionized water periodically. The plates were examined daily under a stereoscopic binocular microscope and if growth was observed then the baits were selected at random from each petri plate and transferred to plates containing SDA medium supplemented with cycloheximide (0.5 mg/ml) and chloramphenicol (0.05 mg/ml). The SDA plates were incubated at room temperature (28±1°C) for further examination.

Identification of isolated fungi
Slide culture technique was adopted for the identification, microscopic studies were done after staining the fungus with the lacto phenol cotton blue and mounting the slide in diphenhydramine mounting medium [23-25].

Preparation of inoculum
B. subtilis, *E. coli* and *E. faecalis,* were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 minutes, pellet was suspended in double distilled water and the cell density was standardized spectrophotometerically (A_{610} nm). The fungal inoculum *A. flavus, A. niger* and *Penicillin natatam* were prepared from 5 to 10 day old culture grown on SDA medium/potato dextrose agar medium. The petri dishes were flooded with 8-10 ml of distilled water and conidia were scraped using a sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

Preparation of SDA
Peptone - 10 g
Dextrose - 20 g
Agar - 20 g
Distilled water - 1000 ml
Preparation of nutrient agar media
Beef extract - 3 g
NaCl - 5 g
Peptone - 5 g
Agar - 15 g
Distilled water - 1000 ml
Preparation of SD broth
Peptone - 10 g
Dextrose - 20 g
Distilled water - 1000 ml
Preparation of nutrient broth
Beef extract - 3 g
NaCl - 5 g
Peptone - 5 g
Distill water - 1000 ml.

Antibacterial activity
The petroleum ether, ethyl acetate and methanol, leaf extracts of *A. indica, C. reflexa, J. curcas* and *O. gratissimum* were tested by disc diffusion method [26]. Different concentrations of the extracts (100 µg/ml) were prepared by reconstituting with methanol, ethyl acetate and petroleum ether. The test microorganisms were seeded into respective medium by spread plate method 10 µl with the 24 hrs cultures of bacteria growth in nutrient broth. After solidification the filter paper discs (5 mm in diameter) impregnated with the plant extracts were placed on test organism-seeded plates. *B. subtilis*, *E. coli* and *E. faecalis* were used for antibacterial test. Streptomycin control used as positive control and methanol, ethyl acetate and petroleum ether used as a negative control the antibacterial assay plates were incubated at 37°C for 24 hrs. The diameter of inhibition zones was measured in mm.

Antifungal activity
The antifungal activity was tested by disc diffusion method [27]. The filter paper discs (5 mm in diameter) impregnated with 100 µg/ml concentrations of the extracts were placed on test organism-seeded plates. Petroleum ether, ethyl acetate and methanol were used to dissolve the extract and were completely evaporated before application on test organism-seeded plates. Blank disc impregnated with solvent methanol followed by drying off was used as negative control and nystatin (10 µg/disc) used as a positive control. The activity was determined after 72 hrs incubation at 28°C. The diameters of the inhibition zones were measured.

RESULTS AND DISCUSSION

Four fungal species were isolated and identified from a soil sample collected from different places of Jabalpur (Madhya Pradesh). *A. niger* was most frequent 60% followed by *A. flavus* (20%), *P. notatum* (10%), *F. oxysporium* (10%). The colony characteristics of the isolated fungi are given in Table 1.

Microscopic study of the fungi

A. niger (van Tieghem)

Colonies consist of a compact white or yellow basal felt covered by a dense layer of dark-brown to black, conidial heads are large (up to 3 mm, 15-20 µm in diameter), globose dark brown, becoming radiate and tending to split into several loose columns with age conidiphores stipes are smooth walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown often septate metulae.

A. flavus (Link ex Grey)

Colonies were lime green to cream colour. Texture woolly on maturity and were dark brown. Colonies granular, flat, often with radials grooves. Conidial heads are typically radiate later splitting to form loose columns (mostly 300-400 µm diameter) having some heads with phialides borne directly on the vesicle. Conidiophores are hyaline and coarsely roughened often more noticeable near the vesicle conidia are globose to sub-globose (3-6 µm in diameter).

P. notatum (link fries rapid)

Growing, filamentous, velvety texture, cottony texture. Colonies were bluish-grey-green at centre and white at periphery. Reverse plate showed pale yellow coloured pigmentation. microscopically, chain of single celled conidia are produced in basipetal succession from a specialized condiogenous cell called a phialide. In penicillium phialides may be produced singly in groups or from branched metulae, givining a brush-like an appearance.

F. oxysporium (Schlecht)

Microconidia are *Fusarium* with slightly curved pointed at the lip mostly three septet basal cells pedicellate. Colonies growing rapidly, 4.5 cm in 4 days conidiophores are short, single, lateral monophialides in the aerial mycelium, later arranged in densely branched clusters. Macroconidia are fusiform slightly curved, pointed at the tip, mostly three septate, basal cells pedicellate, 23-54 µm × 3-4.5 µm. Microconidia are abdunt, never in the chain, mostly non septate, smooth or rough walled, 5-13 µm.

Antibacterial activity
Antibacterial activity of leaves extract of *A. indica, J. curcas, C. reflexa* and *O. gratissimum* in different solvent tested against *E. coli, B. subtilis, Enterococus faecalies.* The inhibition zone in (mm) against the test microorganism is show in Tables 2-4.

The methanolic extract of *O. gratissimum* inhibited the growth of three bacterial species namely *E. coli, B. subtilis, E. faecalis* with zone of inhibition ranging from 5 mm to 10 mm. The extract of *A. indica* and *C. reflexa* had no activity against the entire test bacteria. The activity of *O. gratissimum* was highest against bacterial strain *E. coli* (8 mm), followed by *B. subtilis* (6 mm) and least against *E. faecalis* (5 mm) (Table 2). Petroleum ether did not show any activity against bacterial

Table 1: Occurrence of fungi in different soil samples

S. No.	Fungal species	Colony characteristics after 3 days of incubation			Frequency	
		Color	Diameter	Texture	Number of occurrence	Total CFU
1	*A. niger*	White or yellow basal felt covered by a dense layer of dark- brown to black conidial heads	15-20 µm	Powdery in texture	12	20
2	*A. flavus*	Yellow-green surface pigmentation	6 µm	Texture woolly	4	20
3	*P. notatum*	Yellow color	3 µm	Velvety texture, cottony	2	20
4	*F. oxysporium*	Dark blue or dark purple	15 µm	Smooth to rough walled	2	20

CFU: Colony forming unit, *A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. notatum: Penicillium notatum, F. oxysporium: Fusarium oxysporium*

Table 2: Activity of methanol extract of some plant leaves against bacteria and fungal isolates

S. No.	Plants species	Inhibition zone in mm						
		Bacillus subtilis	*E. coli*	*Enterococcus faecalis*	*A. niger*	*A.flavus*	*Penicillin notatum*	*Fusarium oxysporium*
1	*A indica*	-	-	-	8 mm	-	-	-
2	*J. curcus*	-	-	-	-	-	-	-
3	*C. reflexia*	-	-	-	-	-	-	-
4	*O. gratissimum*	6 mm	8 mm	5 mm	10 mm	-	-	-

A. indica: Azadirachta indica, J. curcus: Jatropa curcus, C. reflexia: Cuscuta reflexia, O. gratissimum: Ocimum gratissimum

Table 3: Activity of petroleum ether extract of some plants leaves against bacterial and fungal isolates

S. No.	Plants species	Inhibition zone in mm						
		Bacillus subtilis	*E. coli*	*Enterococcus faecalis*	*A. niger*	*A. flavus*	*Penicillium notatum*	*Fusarium oxysporium*
1	*A. indica*	-	-	-	-	-	-	-
2	*J. curcus*	-	-	-	-	-	-	-
3	*C. reflexa*	-	-	-	-	-	-	-
4	*O. gratissimum*	-	-	-	-	-	-	-

B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. notatum: Penicillium notatum, F. oxysporium: Fusarium oxysporium, A. indica: Azadirachta indica, J. curcus: Jatropa curcus, C. reflexia: Cuscuta reflexia, O. gratissimum: Ocimum gratissimum

Table 4: Activity of ethyl acetate extract of some plants leaves against bacterial and fungal isolates

S. No.	Plants species	Inhibition zone in mm						
		B. subtilis	*E. coli*	*E. faecalis*	*A. niger*	*A. flavus*	*P. notatum*	*F. oxysporium*
1	*A. indica*	-	-	-	-	-	-	-
2	*J. curcus*	-	-	-	-	-	-	-
3	*C. reflexa*	-	-	-	-	-	-	-
4	*O. gratissimum*	7 mm	9 mm	-	-	-	-	-

B. subtilis: Bacillus subtilis, E. coli: Escherichia coli, E. faecalis: Enterococcus faecalis, A. niger: Aspergillus niger, A. flavus: Aspergillus flavus, P. notatum: Penicillium notatum, F. oxysporium: Fusarium oxysporium, A. indica: Azadirachta indica, J. curcus: Jatropa curcus, C. reflexia: Cuscuta reflexia, O. gratissimum: Ocimum gratissimum

strain (Table 3). Ethyl acetate extract of *O. gratissimum* showed activity against *E. coli* (9 mm) and *B. subtilis* (7 mm) (Table 4).

Antifungal activity

Methanolic extract of *A. indica* (8 mm) and *O. gratissimum* (10 mm) showed significant activity against *A. niger* (Table 2). The other plants extracts did not show any activity against test fungi. Petroleum ether and ethyl acetate extract of all plants did not show any activity against all the fungal strain isolates (Tables 3 and 4).

The present work emphasizes the search of antimicrobial phytochemicals against *E. coli, B. subtilis, E. faecalis, A. niger, A. flavus, P. notatum* and *F. oxysporium.* About 80% of the human population in India is using herbal medicine to cure different kind microbial infection.

Four fungal species were isolated and identified from a soil sample collected from different places of Jabalpur (Madhya Pradesh). *A. niger* was most frequent 60% followed by *A. flavus* (20%), *P. notatum* (10%), *F. oxysporium* (10%). Antibacterial activity of leaves extract of *A. indica, J. curcas, C. reflexa* and *O. gratissimum* were tested against *E. coli, B. subtilis, E. faecalis.*

Results obtained during assay with plant extracts from *A. indica, O. gratissimum, J. curcas* and *C. reflexa* leaves showed their inhibitory effect against some fungal and bacterial isolates.

O. gratissimum shows highest activity against *A. niger* (inhibition zone 10 mm) followed by *E. coli* (inhibition zone 8 mm), *B. subtilis* (inhibition zone 6 mm) and *E. faecalis* have (inhibition zone 5 mm) in methanol extract *A. indica* ranked second with (inhibition zone 4 mm). Results related to antimicrobial activity in leaf extracts of Neem (*A. indica*) was investigated against human pathogenic bacteria [28].

Antibacterial and antifungal efficacy of leaf and seed extract and seed oil of *J. curcas* was investigated against Gram-positive bacteria [29]. In the case of Gram-negative bacteria, seed extract and seed oil has shown some moderate activity whereas, no activity was observed in leaf extract.

J. curcas and C. reflexa showed no activity in methanol extract as already tested antibacterial and antifungal efficacy of leaf and seed extract and seed oil of *J. curcas* against Gram-positive and Gram-negative bacteria. Their results showed no activity in leaf extract [30].

The petroleum ether extract of all the plants did not show any activity against all the test microorganisms.

In ethyl acetate extract is the same case as in methanol only *O. gratissimum* showed well defined activity against *E. coli* and *B. subtilis* with inhibition zone (9 mm, 7 mm respectively) and the *A. indica*, *J. curcas* and *C. reflexa* did not show any activity. Previous reports shows *Ocimum sanctum* exhibited antifungal activity against all the fungi [30], who screened barks of 30 plants species against *Microsporum gypsum* and Trichophyton mentagrophytes, *O. sanctum* was found to possess absolute toxicity. This could suggest that probably certain photochemical exhibit their antimicrobial action only with other phytoconstituents in a synergistic way. Antimicrobial activity of the methanolic extract of *O. gratissimum* and *A. indica* ethyl acetate extract of *O. gratissimum* showed inhibitory activity against *E. coli*, *B. subtilis* and *E. faecalis*. As already *Ocimum tenuiflorum* was tested against selected Gram-positive, Gram-negative bacterial and fungal strains [31]. The activity was performed against common pathogenic bacteria and fungal strains. In their study, the methnolic and ethyl actate extracts of *O. tenuiflorum* were found to be more or less active against almost all tested pathogenic.

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