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Evaluation of Antimicrobial activity of Various Medicinal Plants Extracts of Latur Zone against Pathogens

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ABSTRACT- The present study was planned to study the antimicrobial activity of different plant extract against selected microorganisms. The plants used in the present study were *Ocimum sanctum* (Tulsi), *Withania somnifera* (Ashwgandha), *Santalum album* (Chandan), Aloe vera (Aloe *barbadensis*), and shatavari (*Asparagus racemosus*). The extract from the leaves of these plants (are) used in malaria, bronchitis, gastric disorders, cough, cold etc. To test efficiency of some common plants extract against *E. coli, Salmonella typhi, Proteus vulgaris, Staphylococcus aureus.* Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases. The present investigation is therefore, undertaken to test the efficiency of some of the common plant extracts against some plants and human pathogens, i.e. *E. coli* and *S. aureus*. In this project work, we studied the different parts of medicinal plants of Latur, Osmanabad region used for curing different type of diseases specially skin diseases. Some plants have active components which show antimicrobial activity. These Herbal plants are beneficial to human being in therapeutic practice. Skin diseases are difficult conditions to live with, to save the very least. Though some skin diseases may cause minimal discomfort, the visual effects of the conditions can cause significant self esteem and confidence issues. The majority of skin diseases cause scarring or disfigurement. Skin diseases run the gambit from barely noticeable to fatal.

Key-words- Medicinal plants, Antimicrobial activity, Antifungal activity

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INTRODUCTION

The use of natural products with therapeutic properties has a long history whereas plant, animal, and mineral products were the main source of medicines [1]. Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals and plants. Systematic screening of them may result in the discovery of novel effective antimicrobial compounds [2]. Plants can possess antimicrobial natural products to protect themselves from microbial infection and deterioration [3].

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In the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulterations and side effects [4]. In recent years, concern over pathogenic and spoilage microorganisms in foods has increased due to the increase in outbreaks of food borne disease [5]. There are growing interests in using natural antimicrobial compounds, especially extracted from plants, for the preservation of foods. In addition, there are more consumers who tend to question the safety of synthetic additives and would prefer natural foodstuffs [6,7]. There is therefore the need to search for plants of medicinal value. The plant used in the present study was *Ocium santum* (Tulsi), *Withnia somnifera* (Ashwgandha) *Santalum paniculatumi* (Chandan), Aloe-vera, shatavari. [8] The extract from the leaves of these plants used in Malaria, Bronchitis, Gastric disorders, Cough, cold etc. In recent years more attention has been given to no chemical systems for seed treatment to protect them against seed-borne pathogens. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds.

[9] Below is a list of some of the nastiest skin diseases which are fatal eczema, psoriasis, plaques and rashes on skin, scleroderma, and herpes gladiatorum. Wound healing is an important biological process involving tissue repair and regeneration. The skin disease curing activities of plants have since been explored. The significant successes recorded have led to investigation into medicinal plants with a view to confirming these acclaimed properties. In this study we record the different parts of plants of India used for curing skin disease containing some active principles or components that are antimicrobial in function. In this way we have made an attempt to give an insight into the different parts of herbs having potential use in different skin disease, which could be beneficial in therapeutic practice.

MATERIALS AND METHODS

PLANTS USED

Different plants and their extract where collected to check their antimicrobial activity against different microorganism. In this study 19 plants where used. Leaf extract of different plant where collected from different region of Latur and live plants were also maintained in pots.

Table 1: Information about different medicinal plants

S. No.	Common name	Family	Botanical name	Plant part used
1.	Tulsi	*Labiatae*	*Ocimum sanctum*	Leaves, stem
2.	Neem	*Meliaceae*	*Azadiracta indica*	Leaves, stem
3.	Adulsa	*Acanthaceae*	*Adhatida vasica*	Leaves,
4.	Gudmar	*Asclepiadaceae*	*Gymnema sylvestre*	Leaves,
8.	Chandan	*Santalaceae*	*Santalum paniculatum*	Leaves
9.	Jakhamjodi	*Asteraceae*	*Tridax procumbens*	Leaves
10.	Shatavari		*Asparagus Racemosus*	Leaves
11.	Akarkara		*Anacyclus pyrethrum*	Leaves
12.	Ashwagandha	*Solanaceae*	*Withania somnifera*	Leaves
14.	Karanj		*Pongamia pinnata*	leaves
15.	Nivdung	*Cactaceae*	*Opuntia*	Leaves
16.	Prickly pears	*Cactaceae*	*Opuntia*	Leaves, fruits
17.	Ashoka	*Sillago indica*	*Saraca indica*	Bark, flowers
18.	Unknown 1			Leaves
19.	Unknown 2			leaves

Preparation of Plant Extracts

The plant materials (leaves) were washed thoroughly with distilled water and alcohol and again washed with sterile distilled water. Plant leaves homogenized (grind) by using mortal and pestal by adding distilled water. 10 ml water was used for 1gm plant materials. Extract filtered by using muslin cloth and used for experiment.

Fig. 1: Extraction sample of different plants

Test Organisms Used

Microorganisms used for this experiment are mostly pathogenic in nature, which were collected from MIT Medical College, and R.S. College Latur, India. Culture of Microorganisms maintained for further work by using slants.

Test organism used: *E. coli, Salmonella typhi, Staphylococcus aureus, Proteus vulgaris*

Fungus used: *Candida albicans, Aspergillus fotidus***,** *Aspergillus niger***,** *Penicillum chrysosoprium*

EXPERIMENTAL PROCEDURE

Well Diffusion method for Antibacterial Activity

This method depend on the diffusion of various extract from a well through a solidified agar layer Petri dish, so that the growth of inoculated microorganism is prevented entirely in circular zone around the prepared well containing plant extract using micropipette.

Prepared nutrient agar plates and spreaded the bacterial culture (*E. coli, Staphylococcus aureus, Salmonella typhi, Proteus vulgaris)* on agar surface medium. Made wells on the surface of agar (6mm) with help of cork borer and added 50µl of plant extract in well. For the diffusion of plant extract in the agar, kept plates in refrigerator for 15min. After the diffusion plates were incubated at 37ºC for 24hr in incubator. Observed the zone of inhibition after incubation period.

Disc Method

Prepared nutrient agar plates and spreaded the bacterial culture (*E. coli, Staphyloccocus aureus, Salmonella typhi,* and *Proteus vulgaris*) on agar surface medium. Made a circular disc (5 mm in diameter) deep in the plant extract, put this disc on agar surface.

For the diffusion of plant extract in the agar, keep plates in refrigerator for 15 min. After the diffusion plates are incubated at 37ºC for 24hr in incubator. Observed zone of inhibition after incubation period.

Agar disk diffusion assay

The Agar disk diffusion method of antimicrobial test was developed in 1940 [10]. The procedure which was accepted by NCCLS and widely used now a days, is a modification of that described by Bauer, Kirby, Sherris and Truck (commonly known as Kirby-Bauer test) [11-12]. The Agar disk diffusion technique has been widely used to assay plant extract for antimicrobial activity [13-15]. In this method, 6 mm sterilized filter papers disks (Whatmann No. 1) are saturated with filter sterilized [16] plant extract of desired concentration.

The impregnated discs are then placed onto the surface of a suitable solid agar medium like Mueller Hinton, Trypton soya agar [17] or Nutrient agar [18].

The media has been pre-inoculated with test organisms. The standard inoculum size is of 1 x 108 CFU/ml of bacteria for inoculating diffusion plates [19] which is equal to McFarland 0.5 turbidity standard. Some researchers impregnate the paper disk with plant extract before putting on the inoculated plates while others prefer after [19].

The drying time of impregnated paper disk varies among researchers from 2 h to overnight under a laminar flow cabinet.

Well Diffusion Method for Antifungal Activity

This method depends on the diffusion of various extract from a well through a solidified agar layer Petri dish, so that the growth of inoculated fungus is prevented entirely in circular zone around the prepared well containing plant extract sing micropipette. Prepared czpadox agar plates and spreaded the fungal culture *(Aspergillus niger, Candida albicans, Penicillum chrysosporium, Aspergillus fotidus*) on the agar surface medium. Made wells on the surface of agar (6 mm) with help of cork borer. And add a 50µl of plant extract in well. For the diffusion of plant extract in the agar, keep plates in refrigerator for 15min. After the diffusion plates are incubated at 27ºC for 24hr in incubator. Observed the zone of inhibition after incubation period.

Disc Method

Prepared czpadox agar plates and spread the fungal culture (*Aspergillus niger, Candida albicans, Penicillum chrysosporium, Aspergillus fotidus)* on agar surface medium. Made a circular disc (5mm in diameter) and deep in the plant extract, put these discs on agar surface. For the diffusion of plant extract in the agar, keep plates in refrigerator for 15min. after the diffusion plates are incubated at 37ºC for 24hr in incubator. Observed zone of inhibition after incubation period.

Agar disk diffusion assay

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The impregnated discs are then placed onto the surface of a suitable solid agar medium like Mueller Hinton [20], czpadox agar [21]. The media has been pre-inoculated with test organisms. The standard inoculum size is of 1 x 108 CFU/ml of fungus for inoculating diffusion plates which is equal to McFarland 0.5 turbidity standard. Some researchers impregnate the paper disk with plant extract before putting on the inoculated plates [21], while to drying time of impregnated paper disk varies among researchers from 2 h to overnight under a laminar flow cabinet [22]. Plates are then incubated for 48 h at 25°C (fungi) [23]. After incubation, zone diameter is measured to the nearest whole millimeter at the point wherein there prominent reduction of 80% growth.

RESULTS AND DISCUSSION

Properties of the plant used in the study are discussed in the introduction. Different plants parts are used in this study. The amount of residue extracted with water is high. The three bacterial species are used for the study *E. coli, S. aureus, S. typhi, and* fungal species are used *Candida albicans,* and *Aspergillus niger. Ocimum sanctum* has more zone of inhibition against *C. albicans* than others and the less zone of inhibition jakamjudi against *A. niger.*

Table 2: Shows the antimicrobial and antifungal activity of plant extracts (zone of inhibition measured in mm)

S. No.	Name of plants	*E. coli*	*S. aureus*	*P. vulgaris*	*C. albicans*	*A. niger*
1	*Ocimum sanctum*	4 5 6 ±0.5,±0.5,±0.1	4 4.5 6 ±0.4,±0.5,±0.5	_	6 7 6 ±0.5,±0.5,±0.1	_
2	*Azadiracta indica*	3.5 4 5 ±0.5,±0.5,±0.1	4.5 5 5 ±0.5,±0.5,±0.2	_	4 4.5 4 ±0.3,±0.5,±0.5	3 4 4.5 ±0.5,±0.5,±0.1
3	*Gymnema sylvestre*	_	4.5 5 6 ±0.5,±0.4,±0.1	_	4 4.5 6 ±0.4,±0.5,±0.5	_
4	*Adhatoda vasica*		6 7 8 ±0.5,±0.4,±0.5	_	5 6 6.5 ±0.5,±0.4,±0.5	_
5	*Anacyclus pyrethrum*	_	_	_	6.5 7 8 ±0.5,±0.4,±0.5	_
6	*opuntia*	_		_	_	7.3 7.5 8 ±0.5,±0.4,±0.5
7	*Pongamia pinnata*	_	_	_	_	_
8	*Santalum paniculatum*	_	6.5 7 8 ±0.5,±0.4,±0.5	_	_	_
9	*Jakhamjodi*	_	_	_	_	3.5 4 5 ±0.5,±0.5,±0.1
10	*Withania somnifera*	_	_	_	_	_
11	*Berberis aristata*	_	_	_	_	_
12	*Aloevera barbadensis*	_	_	_	_	_
13	*Nivdung*	_	_	_	_	_
14	*Saraca indica*	_	_	_	_	_

15	Unknown 1	–	–	–	–	–
16	Unknown 2	–	–	–	–	–
17	Mixed culture	10 11 10 ±0.5,±0.5,±0.5	11 11 12 ±0.4,±0.5,±0.5	6.5 7 7.5 ±0.1,±0.5,±0.5	10 11 12 ±0.5,±0.5,±0.5	9 10 11 ±0.5,±0.4,±0.5
18	Control	11.5 11 11 ±0.5,±0.5,±0.4	12 13 13 ±0.5,±0.5,±0.5	8 8 9 ±0.3,±0.5,±0.5	12 13 12 ±0.5,±0.5,±0.5	11 11 11 ±0.4,±0.5,±0.5

Fig. 2: Zone of inhibition *Ocimum sanctum* against *C. albicans*

Fig. 3: Zone of inhibition of *Azadiracta indica* against *S. aureus*

Fig. 4: Zone of inhibition of *Adhatoda vasica* against *S. aureus*

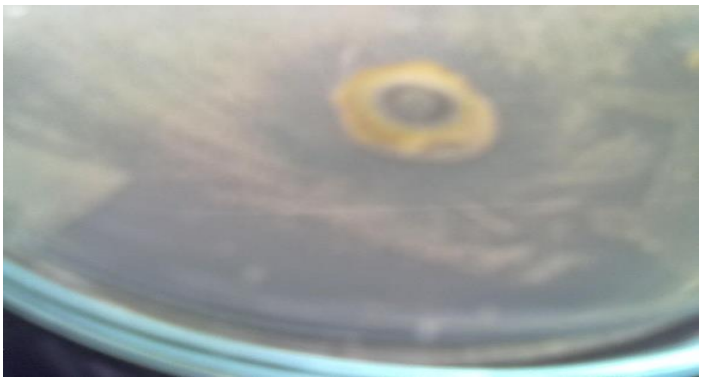

Fig. 5: Zone of inhibition of *Gymnema sylvestre* against *E. coli*

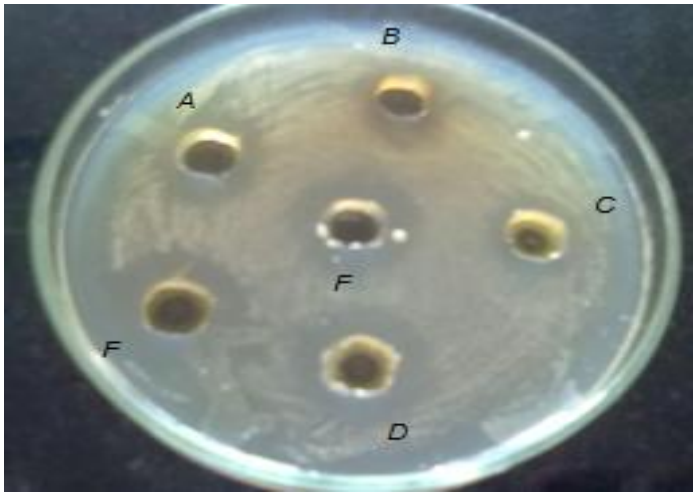

Fig. 6: Zone of inhibition of (A) *Ocimum sanctum*, (B) *Azadirachta indica*, (C) *Alove vera barbadenesis*, (D) *Gymnema sylvestre*, (E) *Santalum album*, (F) *Anacyclus pyrethrum* against *S. aureus*

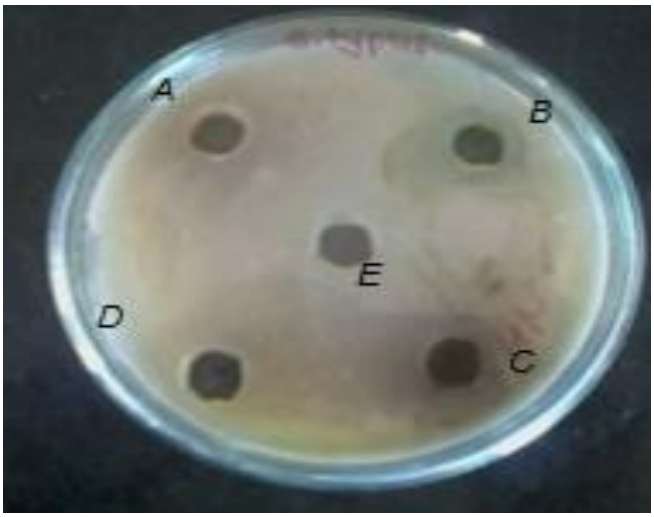

Fig. 7: Zone of inhibition of (A) *Ocimum sanctum*, (B) *Azadirachta indica*, (C) *Alove vera barbadenesis*, D) *Gymnema sylvestre*, (E) *Santalum album*, (F) *Anacyclus pyrethrum* against *S. typhi*

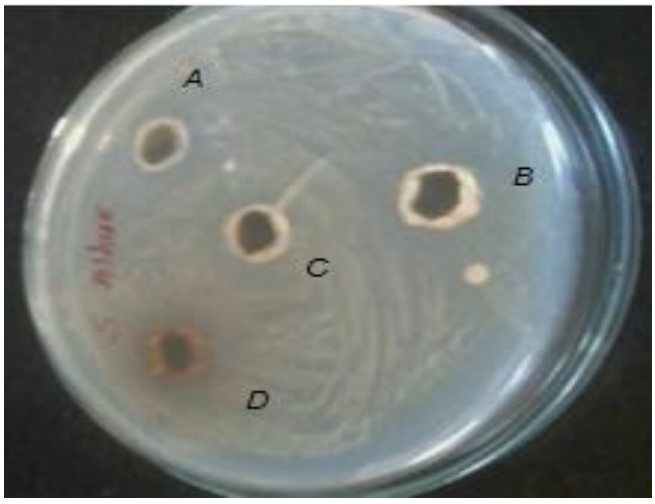

Fig. 8: Zone of inhibition of (A) *Santalum album*, (B) *Alove vera barbadenesis*, (C) *Azadirachta indica* (D) *Ocimum sanctum* against *S. aureus*

Fig. 9: Zone of inhibition of *Ocimum sanctum* against *S. typhi*

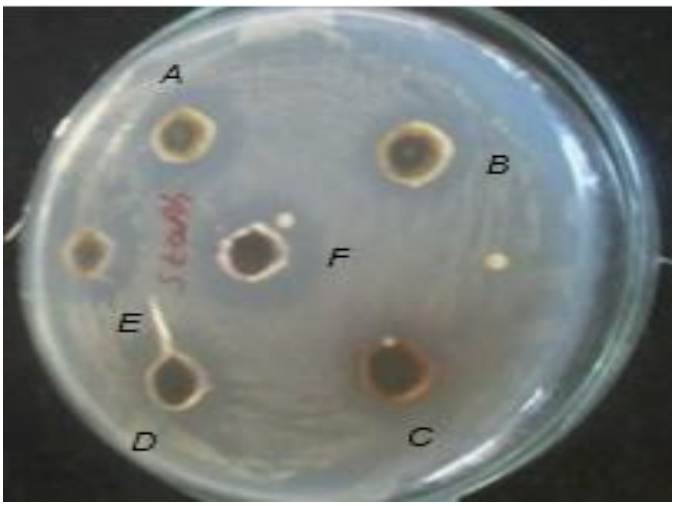

Fig. 10: Zone of inhibition of (A) *Ocimum sanctum*, (B) *Azadirachta indica*, (C) *Alove vera barbadenesis*, (D) *Gymnema sylvestre*, (E) *Santalum album*, (F) *Anacyclus pyrethrum* against *S. aureus*

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