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RESEARCH ARTICLE

Screening of Indian Medicinal Plants and Their Potentials as Antimicrobial Agents.

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Abstract

Methanol extracts of certain Indian Medicinal Plants Cinnamomum zeylanicum, Cinnamomum tamala, Withania somnifera, Myristica fragrans aril, Myristica fragrans kernel & Carum copticum were examined for their anti-microbial potentials against selected bacteria and fungi. The purpose of screening is to justify, authenticate and validate the use of Indian Medicinal Plants in ethno-medicinal or folklore as traditional treasure to cure various ailments. In present investigations attempts were made to screen the Indian Medicinal Plants as antibiotics. The extracts were tested against selected test bacteria and fungi as antimicrobial assay through disc diffusion assay where standard tetracycline is used and solvent Dimethyl sulfoxide as control. Indian Medicinal Plants have a traditional background that they have potentials to use as antimicrobial agents. The results showed that all the extracts possess good antimicrobial activity against selected test bacteria and intermediate against fungus. The present results therefore offer a scientific basis for traditional use of Methanol extracts of Cinnamomum zeylanicum, Cinnamomum tamala, Withania somnifera, Myristica fragrans aril, Myristica fragrans kernel & Carum copticum. Further, almost all the selected plants have also possessed antimicrobial potentials against all test bacteria and fungi which explains that their use in daily life will generate a resistant or immunity to fight against microorganisms.

Introduction:-

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Ayurveda is ancient health care system and is practiced widely in India, Srilanka and other countries (Chopra and Doiphode, 2002). Ayurveda system of medicine use plants to cure the ailments and diseases. Despite the availability of different approaches about for the discovery of therapeutically, natural products still remain as one of the best reservoir of new structural types. They are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds (Cowan, 1999). In modern time plants have been sources of analgesics, anti-inflammatory, anti-neoplastic drugs and medicine for asthma, anti arrhythmic agents and anti hypertensive.

Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001).

In last three decades numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug resistant pathogens (Bandow et al., 2003). In general, bacteria

have the genetic ability to transmit and acquire resistance to drugs (Cohen,1992). According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs (Santos et al., 1995).

Antibiotic resistance has become a global concern (Westh et al., 2004). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various sources like the medicinal plants. Search for new antibacterial agents should be continued by screening many plant families. Recent work revealed the potential of several herbs as sources of drugs. The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics.

Traditional healing systems around the world that utilize herbal remedies are an important source for the Screening of Indian medicinal plants and their potentials as antimicrobial agent's discovery of new antibiotics. Some traditional remedies have already produced compounds that are effective against antibiotic resistant strains of bacteria (Kone et al., 2004). The results of this indicate the need for further research into traditional health system. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity. The need of the hour is to screen a number of medicinal plants for promising biological activity.

Therefore, in present project attempts have been made to medicinal plants Cinnamomum zeylanicum, Cinnamomum tamala, Withania somnifera, Myristica fragrans aril, Myristica fragrans kernel & Carum copticum each belonging to different families were evaluated for antibacterial potentials. Further, all the selected medicinal plants were used to justify and authenticate on scientific basis where antimicrobial characters will be aid as markers to characterize these drugs from their adulterants. These biomarkers can be used further for formation of Indian Pharmacopoeia.

Materials and Methods:-

Collection of Plant Material:-

Plant samples Cinnamomum zeylanicum (Bark), Cinnamomum tamala (Leaf), Withania somnifera (Root), Myristica fragrans aril, Myristica fragrans kernel & Carum copticum (Seed) were collected from various tribes living in tribal pockets of Mt. Abu, arid zone of Rajasthan. These plants were used by these tribes in their daily lives to cure various ailments and few from Chunnilal Attar Ayurvedic Store, Ghat Gate, Jaipur in the month of May, 2012. Identification: All the samples were authenticated and were given identification number Cinnamomum zeylanicum , Cinnamomum tamala, Withania somnifera, Myristica fragrans aril, Myristica fragrans kernel & Carum copticum These samples were authenticated and submitted in Ethnomedicinal Herbarium, Centre of Excellence funded by DST, MGiaS, Jaipur (Rajasthan).

Sources of test organisms:-

Four bacterial strains & two fungal strains were used in this study, gram-positive *Staphylococcus aureus (MTCC 98)* and gram-negative *Escherichia coli (MTCC 1687), Salmonella typhemerium (MTCC 3224) & Pseudomonas aeruginosa (MTCC 1688),* Yeast *Candida albicans (MTCC 227)* & Mould *Aspergillus niger (MTCC 281)* both fungi and bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh.

Culture of test microbes:-

For the cultivation of bacteria, Nutrient Agar Medium was prepared by using 20 g Agar, 5 g Peptone, 3 g beef extract and 3 g NaCl in 1 L distilled water and sterilized at 15 lbs pressure and 121°C temperature for 25-30 min. Agar test plates were prepared by pouring approximately 15 mL of NAM into the Petri dishes under aseptic conditions. A saline solution was prepared (by mixing 0.8% NaCl) in distilled water, followed by autoclaving and the bacterial cultures were maintained on this medium by regular sub-culturing and incubation at 37°C for 24-48 h.
To prepare the test plates, in bacteria, 10-15 mL of the respective medium was poured into the Petri plates and used for screening. For assessing the bactericidal efficacy, a fresh suspension of the test bacteria was prepared in saline solution from a freshly grown Agar slant.

Preparation of powder:-

All plant material were collected and kept in an oven at 70'C overnight to dry. These were later grounded into powdery form and sieved to obtain fine sample. The powder collected was kept in a polythene bag in the laboratory prior to use**.**

Preparation of test extracts:-
Powder (50 g) of all the plant materials were successively soxhlet extracted with 50% Methanol. Later, each of the homogenates was filtered and the residue was re-extracted twice for complete exhaustion, the extracts were pooled individually. Each filtrate was concentrated to dryness in vitro and re-dissolved in DMSO at the concentration of 100 mg/ml, 200 mg/ml, 500 mg/ml.

Preparation of antimicrobial disc:-
Sterile discs were prepared from Whattman No. 1 paper (6 mm in diameter) and used for the preparation of antimicrobial disc. The extracts of the medicinal plants were incorporated to the sterile disc. Each sterile disc was incorporated individually dip in respective concentration of extract and the discs were allowed for air–drying.

Bactericidal assay:-
For both, bactericidal in vitro Disc diffusion method was adopted (Gould and Bowie, 1952), because of reproducibility and precision. The different test organisms were proceeded separately using a sterile swab over previously sterilized culture medium plates and the zone of inhibition were measured around sterilized dried discs (6 mm in diameter), its negative control (DMSO) and reference drugs (standard disk) separately. Such treated discs were air-dried at room temperature to remove any residual solvent, which might interfere with the determination, sterilized and inoculated. These plates were initially placed at low temperature for 1 h so as to allow the maximum diffusion of the compounds from the test disc into the agar plate and later, incubated at 37°C for 24 h in case of bacteria, after which the zones of inhibition could be easily observed. Five replicates of each test extract were examined and the mean values were then referred.

Table 1 Antibacterial activity of Standard antibiotics

Name of Microorganisms	Zone of inhibition (diameter) in mm							
	Cr	**Cx**	**F**	**Va**	**Ak**	**Cf**	**Fr**	**DMSO**
Staphylococcus aureus	32	21	22	16	31	34	22	-
Bacillus cereus	-	-	17	-	23	28	-	-
Escherichia coli	20	-	19	18	21	36	18	-
Pseudomonas aeruginosa	-	-	17	-	20	27	-	-

Cr = Cephaloridine, Ak = Amikacin, Cx = Cloxaccilin, Cf = Ciprofloxacin, F = Framycetin, Fr = Furozolidone, Va = Vancomycin, DMSO= Dimethyl sulfoxide

Table-2: Zone of inhibition of Methanolic plant extract of *Cinnamomum zeylanicum*

Pathogen/Conc.	**100(mg/ml)**	**200(mg/ml)**	**500(mg/ml)**
E. coli	8 mm	11 mm	12 mm
S. typhemerium	9 mm	10 mm	11 mm
P. aeruginosa	8 mm	9 mm	10 mm
S. aureus	8 mm	11mm	12 mm
C. albicans	No Zone	8mm	10 mm
A. niger	No Zone	No Zone	No Zone

Table-3: Zone of inhibition of Methanolic plant extract of *Cinnamomum tamala*

Pathogen/Conc.	**100(mg/ml)**	**200(mg/ml)**	**500(mg/ml)**
E. coli	10 mm	13 mm	14 mm
S. typhemerium	8 mm	10 mm	11 mm
P. aeruginosa	No Zone	No Zone	No Zone
S. aureus	10 mm	11mm	12 mm
C. albicans	No Zone	8 mm	10 mm
A. niger	No Zone	No Zone	10 mm

Table-4: Zone of inhibition of Methanolic plant extract of *Withania somnifera*

Pathogen/Conc.	**100(mg/ml)**	**200(mg/ml)**	**500(mg/ml)**
E. coli	No Zone	No Zone	7 mm
S. typhemerium	No Zone	No Zone	8 mm
P. aeruginosa	No Zone	No Zone	7 mm
S. aureus	No Zone	No Zone	8 mm
C. albicans	No Zone	No Zone	No Zone
A. niger	No Zone	No Zone	No Zone

Table-5: Zone of inhibition of Methanolic plant extract of *Myristica fragrans* aril

Pathogen/Conc.	**100(mg/ml)**	**200(mg/ml)**	**500(mg/ml)**
E. coli	No Zone	8 mm	11 mm
S. typhemerium	No Zone	7 mm	10 mm
P. aeruginosa	No Zone	8 mm	10 mm
S. aureus	8 mm	11mm	12 mm
C. albicans	No Zone	No Zone	7 mm
A. niger	No Zone	No Zone	8 mm

Table-6: Zone of inhibition of Methanolic plant extract of *Myristica fragrans* kernel

Pathogen/Conc.	**100(mg/ml)**	**200(mg/ml)**	**500(mg/ml)**
E. coli	8 mm	11 mm	12 mm
S. typhemerium	No Zone	No Zone	8 mm
P. aeruginosa	No Zone	No Zone	7 mm
S. aureus	9 mm	11mm	12 mm
C. albicans	No Zone	No Zone	7 mm
A. niger	No Zone	No Zone	8 mm

Table-7: Zone of inhibition of Methanolic plant extract of *Carum copticum*

Pathogen/Conc.	100(mg/ml)	200(mg/ml)	500(mg/ml)
E. coli	9 mm	12 mm	14 mm
S. typhemerium	8 mm	10 mm	12 mm
P. aeruginosa	9 mm	11 mm	12 mm
S. aureus	8 mm	10mm	12 mm
C. albicans	No Zone	8 mm	10 mm
A. niger	No Zone	8 mm	12 mm

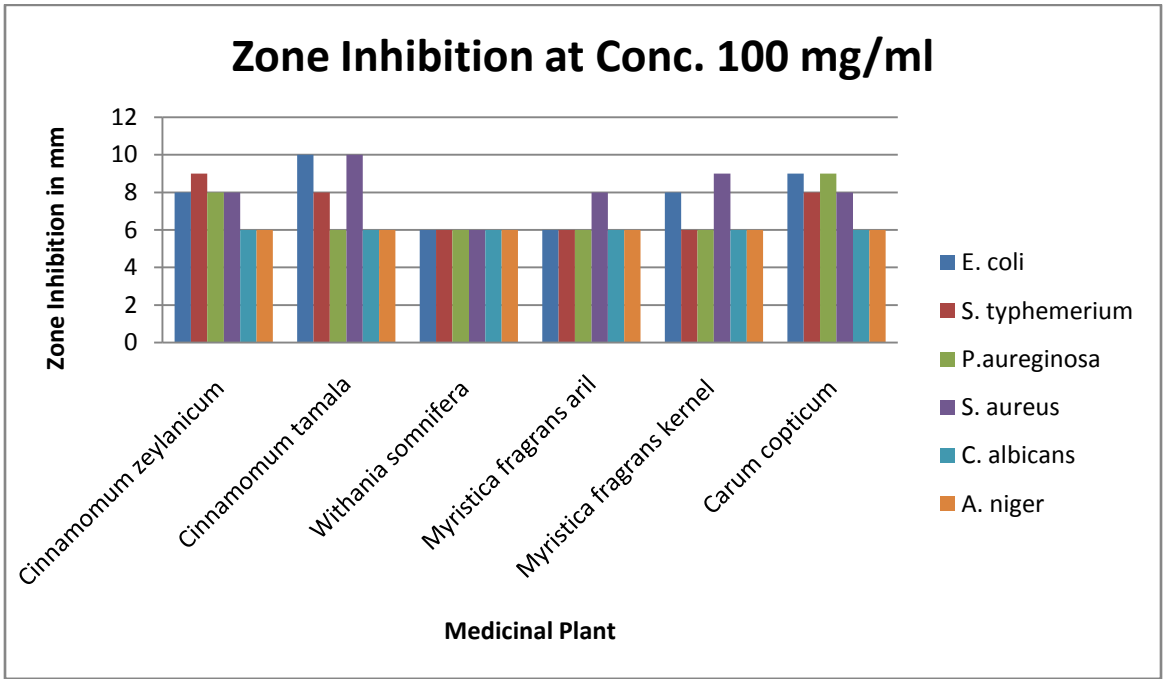
Results and Discussion:-

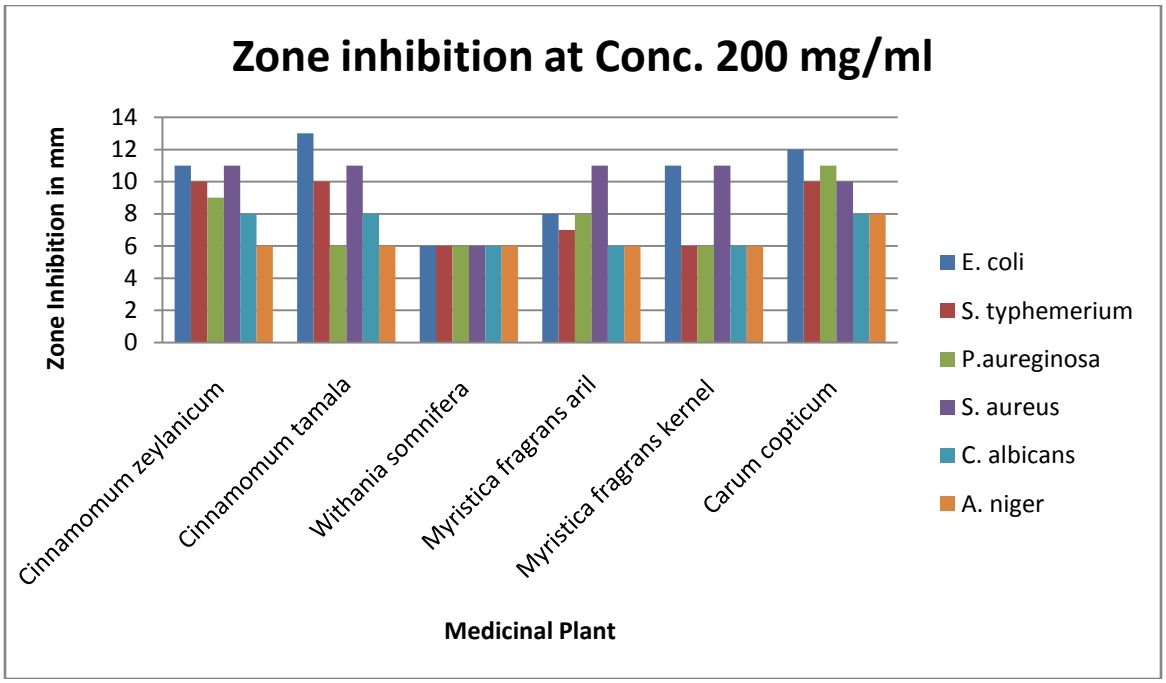
Results:-

The profile of six medicinal plants used in present investigation. The results of antimicrobial activity of the crude extracts of Selected Indian Medicinal Plants (*Cinnamomum zeylanicum, Cinnamomum tamala, Withania somnifera, Myristica fragrans aril, Myristica fragrans kernel & Carum copticum*) showed good antimicrobial activity against selected test bacteria and intermediate against fungi. Overall, this Methanol extract showed appreciable activity against selected test bacteria and fungi and hence, it justifies their use in our traditional system of medicine to cure various diseases.

Table 1: Antimicrobial activity against antibiotics only *S. aureus* shows sensitivity against all seven antibiotics (Cr = Cephaloridine, Ak = Amikacin, Cx = Cloxaccilin, Cf = Ciprofloxacin, F = Framycetin, Fr = Furozolidone, Va = Vancomycin, DMSO= Dimethyl sulfoxide)

Table 2: Methanol extract of *Cinnamomum zeylanicum* the antibacterial activity against selected test bacteria showing good inhibition zone. The Methanol extract have the potential to make inhibition zone against *Pseudomonas aeruginosa* (IZ=8mm), *Salmonella typhemerium* (IZ=9mm), *E. coli* (IZ=8mm), *Staphylococcus aureus* (IZ=8mm) at conc. 100mg/ml & in antifungal activity the inhibition zone against *Candida albicans* is 8mm at conc. 200mg/ml.





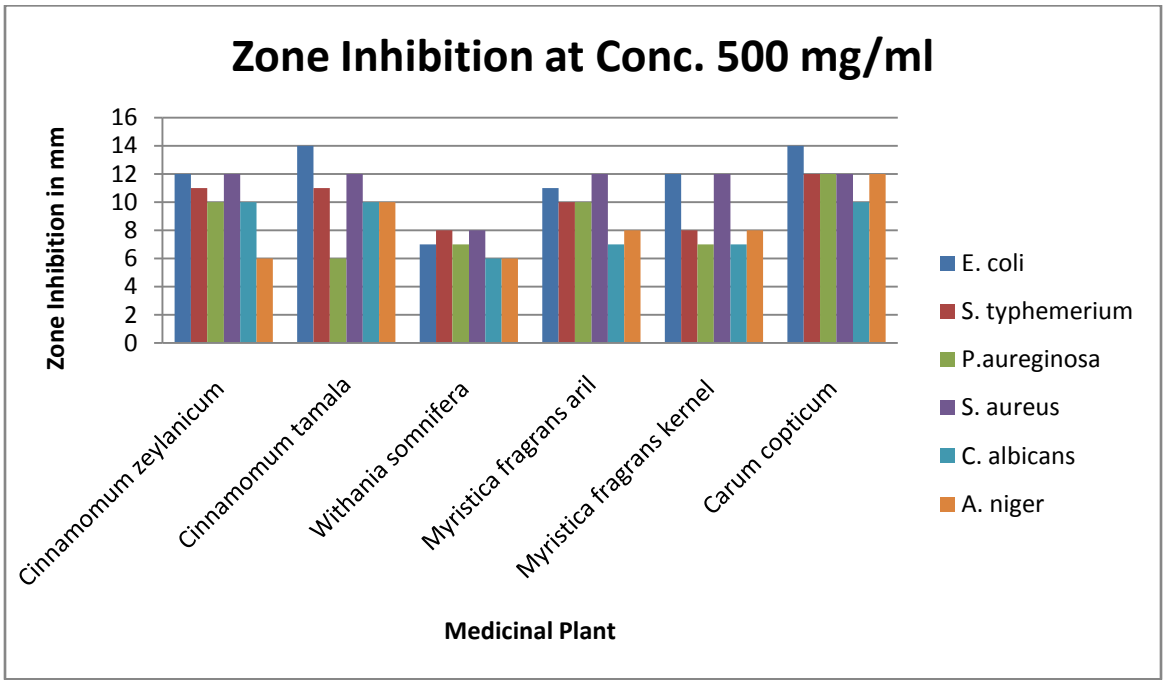


Table 3: The extract of *Cinnamomum tamala* showed no activity against *Pseudomonas aeruginosa* and showed against *Staphylococcus aureus & E. coli*, Showed activity against Fungus at conc. 500mg/ml.

Table 4: Methanol extract of *Withania somnifera* the antibacterial activity against selected test bacteria showing poor inhibition zone. The Methanol extract have the potential to make inhibition zone against *Pseudomonas*

aeruginosa (IZ=7mm), *Staphylococcus aureus* (IZ=8mm), *E. coli* (IZ=7mm), *Salmonella typhemerium* (IZ=8mm) at conc. 500mg/ml and not show antifungal activity.

Table 5: The Methanolic extract of *Myristica fragrans* aril results showed that the given test extracts activity at conc. 200mg/ml.

Table 6: The Methanolic extract of *Myristica fragrans kernel* show antibacterial activity against selected test bacteria showing good inhibition zone. The Methanol extract have the potential to make inhibition zone against *Pseudomonas aeruginosa* (IZ=7mm), *C. albicans & A. niger* at 500mg/ml conc., *Staphylococcus aureus* (IZ=9mm), *E. coli* (IZ=8mm) at conc. 100mg/ml.

Table 7: The Methanolic extract of *Carum copticum* show good antibacterial activity against bacteria. The extract have the potential to make inhibition zone against *Pseudomonas aeruginosa* (IZ=9mm), *Staphylococcus aureus* (IZ=8mm), E. coli (IZ=9mm), *Salmonella typhemerium* (IZ=8mm) at conc. 100mg/ml & in antifungal activity the inhibition zone against *Candida albicans, Aspergillus niger* is10mm, 12mm at conc. 500mg/ml.

Discussion:-

Use of ethno-pharmacological knowledge is one attractive way to reduce empiricism and enhance the probability of success in new drug-finding efforts. Validation and selection of primary screening assays are pivotal to guarantee sound selection of extracts or molecules with relevant pharmacological action and worthy following up. The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections. The present results therefore offer a scientific basis for traditional use of Methanol extracts *Cinnamomum zeylanicum, Cinnamomum tamala, Withania somnifera, Myristica fragrans aril, Myristica fragrans kernel & Carum copticum*. Further, more or less all the selected Indian Medicinal Plants have also possessed antimicrobial potentials against all test bacteria and fungi which explains that their use in daily life will generate a resistant or immunity to fight against microorganisms.

Methanolic extracts of certain Indian Medicinal Plants showed promising antimicrobial potentials against selected test bacteria and fungi. The main aim of these studies is to validate and authenticate the antimicrobial potentials of certain plants and simultaneously, justify their use in the daily diet to cure mankind from certain ailments.

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