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RESEARCH ARTICLE

ANTIBACTERIAL SCREENING OF CERTAIN TRADITIONALLY USED INDIAN MEDICINAL PLANTS AT VIDISHA DISTRICT, MADHYA PRADESH, INDIA.

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Abstract

Ethanolic and aqueous extracts of 10 Indian medicinal plants traditionally used in medicine at Vidisha district were studied for their antimicrobial activity against human pathogenic bacteria of clinical origin. Of these, 09 ethanolic extracts and 08 aqueous extracts of medicinal plant showed varied levels of antibacterial activity against one or more tested human pathogenic bacterial strains. Overall, broad-spectrum antibacterial activity was observed in 05 Indian medicinal plants (both ethanolic and aqueous extracts) against all test microorganisms (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Streptococcus pneumoniae*).

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Introduction:-

The discovery, development, and clinical use of antibiotics during the 20th century have decreased substantially the morbidity and mortality from bacterial infections. The antibiotic era began with the therapeutic application of sulfonamide drugs in the 1930s, followed by a golden period of discovery from approximately 1945 to 1970, when a number of structurally diverse, highly effective agents were discovered and developed. However, since the 1980s the introduction of new agents for clinical use has declined, reflecting both the challenge of identifying new drug classes and a declining commitment to antibacterial drug discovery by the pharmaceutical industry. The same period with a reduced rate of introduction of new agents has been accompanied by an alarming increase in bacterial resistance to existing agents, resulting in the emergence of a serious threat to global public health. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics (**Chopra et al., 1997**).

Today, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. However, rapid emergence of antimicrobial resistance among pathogenic microorganisms has led to a renewed search for new antimicrobial agents. Severe infections caused by bacteria that are resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century (**Alanis, 2005**). Infectious diseases are the world's leading cause of premature deaths, killing almost 60,000 people per day despite remarkable advances in Medical research and treatment during the 20th century, infectious diseases remain among the leading cause of death worldwide. Of these, nosocomial infections comprise about 5 to 10% (**Culver et al., 1985**). It has been estimated that one third of all nosocomial infections may be preventable and are frequently caused by organisms acquired within the hospital environment (**Hughes, 1988**). In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (**Piddock and Wise, 1989; Mulligen et**

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al., 1993; Robin et al., 1998; Omololu et al., 2011). The most resistant bacteria causing important community acquired infections include methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Staphylococcus aureus* (VRSA), vancomycin-resistant *Enterococcus* (VRE), extended spectrum-lactamase (ESBL) producing bacteria such as *E. coli* and *Klebsiella* sp. and multiple drug resistant *Mycobacterium tuberculosis* (MDR-MTB).

Infectious disease caused by bacteria, viruses, fungi and parasites are still a major threat to public health, despite the tremendous progress in human medicine (**Cosa *et al.* 2006**). Such situation stimulates the development of new anti-microbial agents in order to treat the infectious disease in an effective manner. So this matter continued to an era to identify the potential antimicrobial agent from the natural resources. The edible plants that used for traditional medicine contain a wide range of substance that can be used to treat abundant of infectious disease with reduced side effects (**Duraipandiyan *et al.* 2006**).

In India, medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. In the traditional medicinal system of India, Rigveda mentions 67 plants having therapeutic effects, Yajurveda lists 81 plants and Atharveda have 290 plants (**Nabachandra and Manjula, 1992**) besides this the different systems of medicine practiced in India, Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases.

In the present scenario of emergence of multiple drug resistance to human pathogenic organisms, this has necessitated a search for new antimicrobial substances from other sources including plants. Therefore, it is imperative to search for new, efficacious and safe antibiotics from natural sources to combat the menace of drug-resistant infections. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. In the present study, we have selected 10 Indian medicinal plants to be screened against human pathogenic bacteria.

Material And Methods:-

Plant Material

Ten authenticated plants namely Beta vulgaris L., Coriandrum sativum L., Emblica officinalis Gaerth., Eucalyptus sp., Lantana camara L., Lawsonia inermis L., Mentha arvensis L., Ocimum sanctum L., Syzgium aromaticum L. and Terminalia indica L. were collected different areas of Vidisha district. The taxonomic identities of these plants were confirmed by relevant data and were further identified by a senior taxonomist Dr. S. K. Jain, Dept. of Botany, S. S. L. Jain P. G. College, Vidisha and voucher specimens have been deposited in the Department of Botany, St. Mary's P. G. College, Vidisha. The details of medicinal plants along with their specimen code number are listed in Table 1.

Preparation of Plant Extracts

Plant extracts were prepared by the method of **Harborne (1984)**. Briefly, 100 grams of each powered plant sample was extracted with 100 ml of ethanol and distilled water for ethanolic and aqueous extracts respectively using the Soxhlet apparatus. After extraction, an excess was evaporated under reduced pressure in vacuum evaporator. The dried crude extracts were sterilized overnight by UV radiation and then stored at 4ºC in amber color glass vials until further use.

Preparation of Plants Derived Antibiotic Discs

Both the crude extracts (ethanolic and aqueous) each 100 mg, were dissolved in 1 ml of dimethyle sulphooxide (DMSO) and were filtered by using membrane (pore size 0.47 µm). The discs of 6 mm diameter (Sterile blank, HiMedia, Bombay, India) were impregnated into the concentration of the each extracts. The final impregnated discs used for the sensitivity test were 100 mg $disc^{-1}$. These impregnated discs were dried in incubator at 37 ºC for 18 – 24 hours and after this stored in an amber colour glass bottle at room temperature until further use.

Microorganisms Used

The test organisms were used *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella typhi* and *Streptococcus pneumoniae*. The test bacterial strains were collected and isolated from the Department of Microbiology, Gandhi Medical College, Bhopal, M P., India All the clinical isolates were biochemically and serologically characterized by standard method (**MacFaddin, 1985**).

Antibiotics Resistance of test strains

The antibiotic sensitivity of all the test strains was determined by the standard disk diffusion method of **Bauer *et al*., 1966** against a number of antibiotics. The potency of antibiotics discs are: Ciprofloxacin (05 µg $disc^{-1}$ each), Gentamycin and Streptomycin (10 µg $disc^{-1}$ each), Erythromycin, Chloramphenicol and Vancomycin (30 µg $disc^{-1}$ each). All antibiotic discs were purchased from the Hi-Media, Bombay, India. The details of antibiotics resistance of test strains are listed in Table 2.

Antimicrobial Assay of Plants Derived Antibiotic Discs

For this, the standard disk diffusion method of **Bauer et al., 1966** was used. 0.1 ml of diluted inoculum (10^5 CFU / ml) of test organisms was spread on Muller-Hinton agar plates. The plant derived antibiotics discs were placed on the agar plates. DMSO was used as a control. The plates were incubated at 37ºC for 18 – 24 hours. The antibacterial activity was evaluated by measuring the zone of inhibition (ZOI) in mm against the test organisms.

Results And Discussion:-

Emergence of multidrug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antibiotics or antimicrobial drugs of plant origin. In the present study ethanolic and aqueous extracts of 10 traditionally used Indian medicinal plants have been tested against 05 human pathogenic bacterial strains.

Ethno-botanical data, plant parts used along with their voucher specimen number are given in Table 1. The bacterial susceptibilities to the tested antibiotics are shown in Table 2. Most of the antibiotics were inhibited the growth of at least two bacterial strains accept chloramphenicol that inhibited four bacterial strains. The antibacterial screening of the extracts and their potency was quantitatively assessed by the presence or absence of inhibition zone and zone diameter as given in Table 3. The alcoholic and aqueous extracts were tested, as alcohol was found to be a better solvent for extraction of anti-bacterially active substances compared to water (**Ahmad et al., 1998**). The results of screening are encouraging as out of the 10 plants 09 ethanolic and 08 aqueous extracts showed antibacterial activity against one or more test bacteria.

Ethanolic extracts of 05 medicinal plants namely *Eucalyptus* sp., *L. camara*, *L. inermis*, *S. aromaticum* and *T. indica* inhibited all the test bacteria in broad spectrum. Similarly, 05 aqueous plants extracts namely *E. officinalis*, *L. camara*, *L. inermis*, *S. aromaticum* and *T. indica* inhibited all the test bacteria in broad spectrum. Similar reports on antibacterial activities of Indian medicinal plants were also reported by other workers (**Nimri et al., 1999; Geeta et al., 2001; Farrukh and Iqbal, 2003**).

In the case of test bacteria, the basis for their differences in susceptibility might be due to the differences in the cell wall composition of Gram + ve and Gram - ve bacteria (**Grosvenor et al., 1995**). *B. subtilis* was least sensitive compared to other test bacteria, which may be due to their ability to form highly resistant resting stages called endospores. Drug-resistant strains of bacteria were found to be sensitive to the tested plant extracts. This has clearly indicated that antibiotic resistance does not interfere with the antibacterial action of plant extracts and these extracts might have different modes of action on test organisms.

Major factor limiting the long-term use of antibiotic agents is resistance. Before antibiotics era, many people died of bacterial infections caused by pathogens as *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumonia*. Use, abuse or misuse of antimicrobial agents has encouraged the evolution of bacteria towards resistance that often results in therapeutic failure (**Straut et al., 1995**). Prescribing practice of specific class of antibiotics to certain organisms has been found to play a critical role in development of resistance against that antibiotic (**Metz-Gercek et al., 2009; Costelloe et al., 2010**). Thus, antibacterial resistance findings and understanding are necessary to help minimize the emergence of multi drug resistant organisms by promoting prudent use of antibiotics, for this purpose, the need for general public to be appropriately informed on use of antibiotics has been emphasized (**Euro surveillance editorial team, 2010**).

In this study, most of the plants were also screened previously against other test strains (**Agrawal and Dubey, 2018; Agrawal et al., 2014; Agrawal et al., 2012; Agrawal et al., 2007; Mehmood et al., 1999**) and showed similar results with varying degrees of potency. The difference in potency may be due to the stage of collection of the plant sample, different sensitivity of test strains and method of extraction (**Nimri et al., 1999**).

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Table 1:-Ethno-botanical data of 10 Indian Medicinal Plants.

Botanical name; Family Voucher specimen no.	**Common name**	**Part used**	**Traditional uses (Chopra et al., 1992)**
01. *Beta vulgaris* L.; Chenopodiaceae SMCDB-01/18	Chokunder	Roots	Cooling, diaphoretic.
02. *Coriandrum sativum* L.; Apiaceae SMCDB-02/18	Dhana	Leaves	Antioxidants, diabetes
03. *Emblica officinalis* Gaerth.; Euphorbiaceae SMCDB-03/18	Amla	Fruit	Acrid, cooling, refrigerant diuretic, used in diarrhea, dysentery, anemia, jaundice and cough.
04. *Eucalyptus* sp.; Myrtaceae SMCDB-04/18	Eucalyptus	Leaves	Antiseptic, infections of upper respiratory tract, skin diseases, burns, rheumatism.
05. *Lantana camara* L.; Verbenaceae SMCDB-05/18	Raimunia / Ghaneri	Leaves	Decoction used in malaria, rheumatism.
06. *Lawsonia inermis* L.; Lythraceae SMCDB-06/18	Heena / Mehandi	Leaves	Headache, burning of skin, decoction used for sore throat.
07. *Mentha arvensis* L.; Lamiaceae SMCDB-07/18	Pudina	Leaves	Entire plant is antibacterial, headache, rhinitis, cough, sore throat, colic and vomiting.
08. *Ocimum sanctum* L.; Labiatae SMCDB-08/18	Tulsi	Leaves	Gastric disorder, bronchitis, ear ache, antiseptic, diaphoretic, hepatic affections.
09. *Syzgium aromaticum* L.; Myrtaceae SMCDB-09/18	Laung	Bud	Stimulant, carminative, used in dyspepsia.
10. *Tamarindus indica* L.; Leguminoceae SMCDB-10/18	Imli	Fruit	Gastric and digestion problems, astringent, bacterial skin infections (erysipelas), boils, indigestion, insecticide, antimicrobial, antiseptic, antiviral, liver disorders, nausea and vomiting (pregnancy-related).

Table 2:-Antimicrobial Screening of some antibiotics.

Antibiotics	**Antimicrobial activity**				
	[a]SA	**BS**	**EC**	**ST**	**SP**
[b]Chloramphenicol 30 µg $disc^{-1}$	[d]3+	3+	2+	--	2+
Ciprofloxacin 05 µg $disc^{-1}$	--	--	3+	1+	--
Erythromycin 30 µg $disc^{-1}$	--	1+	1+	--	--
Gentamycin 10 µg $disc^{-1}$	--	1+	1+	--	--
Streptomycin 10 µg $disc^{-1}$	2+	--	--	1+	--
Vancomycin 30 µg $disc^{-1}$	--	--	--	2+	1+
[c]DMSO blank solvent	--	--	--	--	--
Total number of active antibiotics	02	03	04	03	02

[a]SA = *Staphylococcus aureus*, BS = *Bacillus subtilis*, EC = *Escherichia coli*, ST = *Salmonella typhi*, SP = *Streptococcus pyogenes*.

[b] shows antibiotics used for bacteria.

[c]DMSO blank solvent used as a control.

[d]1+, < 10 mm; 2+, < 20 mm; 3+, < 30 mm; 4+, < 40 mm, -- , no zone of inhibition.

Table 3:-Antimicrobial Screening of Ethanolic and Aqueous Extracts of 20 Indian Medicinal Plants.

Plant name	Antimicrobial activity									
	Ethanolic extract					Aqueous Extract				
	[a]SA	BS	EC	ST	SP	SA	BS	EC	ST	SP
01. *Beta vulgaris* L.	[b]2+	--	2+	--	--	--	--	2+	--	--
02. *Coriandrum sativum* L.,	--	--	--	--	--	--	--	--	--	--
03. *Emblica officinalis* Gaerth.	4+	2+	--	3+	2+	3+	2+	--	2+	1+
04. *Eucalyptus* sp.	3+	2+	2+	3+	3+	2+	2+	1+	2+	2+
05. *Lantana camara* L.	2+	2+	2+	3+	2+	1+	1+	1+	2+	1+
06. *Lawsonia inermis* L.	4+	2+	2+	4+	2+	3+	2+	1+	3+	1+
07. *Mentha* arvensis L.	2+	--	--	2+	3+	--	--	--	1+	2+
08. *Ocimum sanctum* L.	2+	--	1+	--	4+	1+	--	1+	--	3+
09. *Syzgium aromaticum* L.	3+	2+	3+	3+	4+	3+	2+	2+	2+	2+
10. *Terminalia indica* L.	3+	2+	2+	3+	2+	2+	1+	1+	2+	1+
Total number of active plants	**09**	**06**	**07**	**07**	**08**	**07**	**06**	**07**	**07**	**08**

[a]SA = *Staphylococcus aureus*, BS = *Bacillus subtilis*, EC = *Escherichia coli*, ST = *Salmonella typhi*, SP = *Streptococcus pyogenes*.

[b]1+, < 10 mm; 2+, < 20 mm; 3+, < 30 mm; 4+, < 40 mm, -- , no zone of inhibition.

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