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To Study Chemical Analysis and Biological Activity of Antimicrobial

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ABSTRACT

Recently many scientists have looked for the new antimicrobial. But search for new antibiotics usually used soils as the source of bacteria and fungi. Spices and herbs have been used for the flavor and aroma of foods. Early cultures have also recognized the value of spices and herbs in preserving foods and for their medicinal value. Scientific experiments since the late 19th century have study the antimicrobial properties of some spices, herbs, and their components[1,2]. Chemotherapy refers to the treatment of disease with chemicals. In current, however the term applies primarily to the treatment of infectious diseases and cancer. When infectious diseases, are used as a referred the term is antimicrobial chemotherapy. Antimicrobial chemotherapy can be used either for prophylaxis (prevention) or treatment (cure) of disease caused by bacteria, fungi, viruses, protozoa or helminthes. The production and use of penicillin in the early 1940s became the basis of the chemotherapy era. Streptomycin was discovered in 1944, and many other antibiotics have since been discovered and put into use.

1-Introduction

Micro-organisms were assigned the name seeds of disease by Lucricues in his book entitled **Dream Nature[3].** Antony Van Leeuwenhock [1632-1725] was the first to see an entirely new word of what is now known as microbe. Hence, he is accredited as the Father of Microbiology.

Man is greatly influenced by the activities of micro-organisms. They are employed in the manufacturing of dairy products, certain food items, in the processing of certain materials for clothing, in the preparation of certain drugs and therapeutic agents, in the manufacture of certain chemicals and in many other ways.

Besides their benefits micro-organisms bring harm to man in many ways[4,5] either by causing disease directly or by affecting their animals and crops. When micro- organisms come in contact with the body surface of the host, they invade the tissue and cause disease if they find suitable conditions for their growth. The causal pathogens of diseases like leprosy, abscess, tetanus diphtheria etc. have been isolated by various microbiologists. In 1979, Henle identified the first virus associated with human cancer. Control of microbial populations is essential to prevent disease transmission [6]. Infection, decomposition, contamination and damage caused by them. The personal comforts of human beings largely depend on the control of their population.

Chemotherapeutic agents used in the treatment of disease are of three types: (i) Natural products. (ii) Chemical substances produced by microorganisms, which include antibiotics (iii) Synthetic chemicals.

The existence of various germs was established by the fermentation experiments of French chemist Louis Pasteur [1922-1829] By his experiments, he confirmed what Spallanzani had realised a century earlier that some kind of fermentation was caused by bacteria or fungi. Turning to human and animal diseases, his observations confirmed that specific micro-organisms cause specific diseases.

Antibacterial drugs

Antibacterial agents are classified as narrow-spectrum and broad-spectrum agents.

Narrow-spectrum agents (such as penicillin G) primarily affect Gram-positive bacteria, while broad-spectrum agents, such as (tetracycline and chloramphenicol), primarily affect both Gram-positive and some Gram-negative bacteria. Some examples were discussed.

(i)- Streptomycin was the first of the aminoglycosides to be discovered and was the second antibiotic used in chemotherapy. Another important use was as part of combination therapy for tuberculosis. It still has some use in combination with penicillin to treat infections of the heart valves (endocarditis) and with tetracycline in the treatment of plague, tularemia and brucellosis.

(ii)-Kanamycin is used in the treatment of septicemia (blood poisoning), meningitis and urinary tract infections caused by gram-negative bacteria Because many organism dre resistant to its effects however, kanamycin is now being replaced by other drugs Gentamicin, tobramycin, netilmicin and amikacin are similar in their range of antimicrobial activity They against infectious caused by staphylococci and gram-negative bacteria including pseudomonas aeruginosa.

For the antibacterial study the following organism have been selected

Bacteria are micro-organisms which are rigid walled, unicellular and microscopic. They form a diverse, heterogenous group which possesses properties common to both simple plants and animals, in varying degrees. Bacteria are classified depending on their morphology, staining, nutrition, etc. Despite immense diversity, on the basis of morphology they can be divided into spherical (coccus), straight rods (Bacillus) and curved or spiral rods (Vibrio, Spirillum). Bacteria can be gram-positive or gram-negative organisms. All cocci except Neisseria are gram-positive and all rod-shaped bacteria except Bacillus, Clostridium, Corynebacterium and Mycobacterium are gram-negative.. Cocci are 1 µm in diameter, length and width of bacilli are 2-5 µm and 05-10 µm but some spirochaetes has length and width 5-20 µm and 0.1-0.2 µm.

The bacteria tested are as follows

1. Bacillus subtilis

The genus Bacillus contains gram-positive, aerobic, and spore-bearing rods. *B. Subtilis* is a saprophyte commonly found in soil, water, dust, and air. Their heat-resistant spores contaminate culture media injection fluids, spoil food, etc. This anthrax causes conjunctivitis, can lead to gangrene and septicemia.

2. Escherichia coli

This is a specific coli form of the intestinal tract. It is particularly important as a urinary tract pathogen and also in appendicitis, peritonitis, cholecystitis, neonatal meningitis, wound infections, septicemia and gastroenteritis in infancy. Bacteremia or endotoxic shock is caused by the ingestion of large numbers of E. coli. It is a Gram-negative bacterium isolated from Escherichia in 1885.

Fungi

The Fungi are one of the three "obvious" Eukaryotic Kingdoms. Along with Animals and Plants, the Fungi make up essentially all of the macroscopic organisms. Fungi are far more abundant than most people imagine, with the number of described species at 70,000 and climbing, and the number of presumed but unknown species somewhere in the neighborhood of 750000.

The fungi tested are as follows:

1-Trichoderma viridae

It is a soil fungus and produces white, yellowish or green colonies when cultured. It is used in the commercial production of the enzyme cellulose. It is an obligate saparobe. It is well known soil inhabitant showing antagonism against several plant pathogenic fungi.

2-Aspergillus sp.

It is a filamentous fungus. Fungi of this genus are common saprophytes and often contaminate food and cultures, causing aspergillosis, aspergilloma, allergic alveolitis, and chronic ear infections.

Microbiology, in the largest sense, is the collective study of bacteria. Mycology, Virology, Parasitology and Immunology. However, here in discussing the antimicrobial activity of the natural product, we will focus specifically on those compounds that are biocidal or inhibitory in nature, antibacterial and antifungal.Though this exclusionary use of the term "microbe. by referring only to bacteria and fungi, ignores countless other "microorganisms" which are important to human health, etc., it is by no means a narrow scope, as it spans perhaps the largest taxonomic range possible to include both the prokaryotic kingdom of Monera (i.e,bacteria) and the eukaryotic kingdom of Fungi

Materials and Methods

Several methods have been used by several workers to determine the antimicrobial activity in vitro.[7-10] They are

a-Turbidimetric method

b-Serial dilution tube technique

c-Diffusion methods-

- Agar strip diffusion method
- Agar cup method
- Agar cup cylinder method
- Replica plate method
- Filter paper disc diffusion plate method

In the present study, the filter paper disc diffusion plate method was employed to evaluate the antimicrobial activity in vitro.

Preparation of media[11,12]

a-For bacteria

The preparation of inoculum for bacteria has been done as follows oxoid culture

broth

Lab Lemco beef extract - 3gm

Peptone - 10 gm

Glucose - 20 gm

Distilled water - 1000 ml

Oxoid nutrient agar medium having the following composition, was used for preparing slants and plates.

Lab Lemco beef extract- 3 gm.

Peptone- 10 gm

Glucose- 20 gm

Agar- 20 gm

Distilled water- 1000 ml.

b-For fungi

Fungal inoculum having the following composition has been prepared

Sliced potato extract -	200 gm
Dextrose -	20 gm
Distilled water-	1000 ml.

Potato dextrose agar (PDA) medium was used for preparing slants and plates.

Sliced potato extract-	200 gm.
Dextrose-	20 gm.
Agar-	20 gm.
Distilled water -	1000 ml

All components were dissolved in hot distilled water, flasks plugged and sterilized by autoclaving at 15 lbs pressure for 30 minutes.

Results and Discussion

The present work deals with in vitro screening of antimicrobial activity of the petroleum ether and alcoholic extracts of selected seeds and prepared heterocyclic compounds. From the results of activity of these compounds, some of these have a pronounced inhibitory effect on selected bacteria and fungi.

Among the plant extracts(tables I and II) it can be said that the alcoholic extract of *T. urgentia* possess good antibacterial activity and Petroleum ether extract of *G.maculata* shows good antifungal activity. These extracts showed very good activity on one particular bacteria ie *Shigella dysentria.*

Antimicrobial activity of thiazolidinones (table III), nitro substituted by phenyl ring shows good activity on all tested organisms. Other derivatives which are substituted by thiazol, show moderate activity on all

fungi especially on *candida albicans*. Oxazol substitution moderate activity on all bacteria and the remaining compounds showed comparitively less zone of inhibition against all tested organisms.

Table I: Antibacterial and Antifungal Activity of Alcoholic Plant of Seed Extracts

Name of the Plant	Antibacterial activity						Antifungal activity					
	B.S		E.C		S.D		T.V		A.S		C.A	
	4%	2%	4%	2%	4%	2%	4%	2%	4%	2%	4%	2%
Tecoma urgentia	10.8	8.0	12.0	10.5	13.0	11.0	18.0	16.0	15.0	12.5	21.5	19.0
Gliricidia maculate	14.0	12.0	10.0	8.0	11.0	10.0	15.0	12.5	11.0	9.0	12.0	9.0
Standard	**28**	**25**	**23**	**20**	**21**	**19**	**25**	**23**	**21**	**19**	**26**	**24**

Table II: Antibacterial and Antifungal Activity of Alcoholic Plant of Seed Extracts

Name of the Plant	Antibacterial activity						Antifungal activity					
	B.S		E.C		S.D		T.V		A.S		C.A	
	4%	2%	4%	2%	4%	2%	4%	2%	4%	2%	4%	2%
Tecoma urgentia	11.0	9.0	12.0	08.0	06.5	14.0	13.0	07.0	08.0	07.5	06.0	05.0
Gliricidia maculate	20.0	17.0	15.0	13.5	20.0	17.5	12.0	11.0	10.0	8.0	14.0	11.0
Standard	**28**	**25**	**23**	**20**	**21**	**19**	**25**	**23**	**21**	**19**	**26**	**24**

Table III: Antibacterial and Antifungal Activity of Thiazolidinones

Name of the	Antibacterial activity			Antifungal activity		
Compoud	B.S	E.C	S.D	T.V	A.S	C.A

	4%	2%	4%	2%	4%	2%	4%	2%	4%	2%	4%	2%
4-(2-phenyl-4-thiazolidinon-3-yl)-6 benzophenone	-	-	8	5	-	-	10	8	6	5	8	6
4-(2-(3" chloro phenyl)-4- thiazolidinon-3-yl)- benzophenone	11	10	13	12	14	12	17	16	10	9	15	12
4-(2-(4" chloro phenyl)-4- thiazolidinon-3-yl)- benzophenone	12	10	13	12	14	13	19	18	12	10	15	12
4-(2-(3", 4" di chloro phenyl)-4-thiazolidinon-3-yl)-benzophenone	14	12	16	14	17	14	20	19	14	12	17	16
4-(2-(3" 4" di methoxy phenyl)-4-9 thiazolidinon-3-yl)- benzophenone	9	8	10	9	12	10	15	13	8	7	13	11
4-(2-(3" hydroxy phenyl)-4-6 thiazolidinon-3-yl)- benzophenone	6	5	9	7	-	-	-	-	11	10	9	7
4-(2-(4 methoxy phenyl)-4-8 thiazolidinon-3-yl)- benzophenone	8	7	11	10	9	8	11	10	8	7	10	8
4-(2-(3" nitro phenyl)-4- thiazolidinon-3-yl)- benzophenone	21	18	20	17	18	16	22	20	16	14	21	20

lidinon-3-yl)-benzophenone											
4-(2-(oxazol)-4-thiazolidinon-3'-yl)-10 benzophenone	13	12	14	12	15	14	-	-	9	8	12
4-(2-(thiazol)-4-thiazolidinon-3-yl)-24 benzophenone	16	14	15	14	17	15	20	18	18	16	25
4-(2-(4-hydroxy .3"-methoxy phenyl)-11 4-thiazolidinon-3-yl)- benzophenone	9	7	12	11	11	9	13	12	12	11	14
Standard 24	**28**	**25**	**23**	**20**	**21**	**19**	**25**	**23**	**21**	**19**	**26**

Conclusion

Activity was determined using prepared thiazolines, pyrazoline 5-ones (two series), isoxazole derivatives and 4% and 2% solutions of alcoholic extracts of selected seeds in petroleum ether, ethylene glycol. 4% and 2% solutions of the standard drugs griseofulvin (for fungi) and streptomycin (for bacteria) were also prepared. Filter paper discs were soaked in standard drug solutions and activity was determined.

From all the compounds tested, it was observed that pet. ether extract of G. maculata thiazole substituted by pyrazoline and nitro group had moderate activities on all the synthetic compounds and could be used as antimicrobial agents.

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