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Phytochemical and Antimicrobial Analysis of Triticum Aestivum and Bauhinia Variegate Corresponding

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Abstract—*Triticum aestivum and Bauhinia variegata were the two plants selected for the study . These are highly potential and very effective plants. They have been exploited from ancient times for their antioxidant, antimicrobial, anticancerous and antidiabetic properties. Phytochemical analysis of the plants was done with various solvents (petroleum ether, methanol, hexane) following the standard protocols for analysis. The results were positive and showed the presence of alkaloids, sterols, phenolic compounds, quinones, anthraquinones, terpenoids and tannins. Antibacterial activity of both the plant extracts were analysed by disc diffusion method against various gram positive and gram negative strains (Salmonella typhi, Staphalococcus aureus, Pseudomonas aeruginosa, E.coli). The zone of inhibition observed was comparable to the standard streptomycin. A mixture of both the plant extracts was also tested for antibacterial activity.*

Keywords*: Phytochemical analysis, Triticum aestivum, Bauhinia variegata, antimicrobial analysis.*

1. INTRODUCTION

Phytochemistry in literal meaning is study of phytochemicals. These are chemicals derived from plants. Phytochemical technique mainly applies to the quality and quantity estimation of various chemical components, such as saponins, alkaloids, volatile oils, flavanoids, anthraquinones. The use of plants as medicines goes back to early man. Certainly the great civilisations of the ancient Chinese, Indians, and North Africans provided written evidence of man's ingenuity in utilising plants for the treatment of a wide variety of diseases[1]. Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins sugar and amino acids. Secondary constituents contain terpenoids and alkaloids[2]. Medicinal plants have antifungal, antibacterial and anti-inflammation activities. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing of various diseases. Infectious diseases are still a major threat to public health, despite the tremendous progress made in human medicine. Contrary to synthetic drugs, antibacterials of plant origin are not associated with many side effects and have an enormous therapeutic potential to treat many infectious diseases[4]. The plants selected for study are *Bauhinia variegata* and wheatgrass *(Triticum aestivum).*

2. MATERIALS AND METHODS

2.1 Extraction procedure

Extraction using Soxhlet apparatus: Take 20gms of sample (*Triticum aestivum* & *Bauhinia variegata)* and 300ml of solvents (like Methanol, Petroleum Ether, Hexane and aqueous) in soxhlet. Run the apparatus according to the boiling temperature of the solvent for 2 hours. After 2 hours collect the extract and go for Rotary evaporator.

Concentration of extract using Rotary evaporator: Take the extract and separate the solvent used from the extract. Remaining entity will be the concentrated extract. Dry the extract, weight the extract and dissolve it in known quantity of DMSO.

2.2 Qualitative Tests For Phytochemicals:

Alkaloids

Mayer's test: Alkaloids give cream colour precipitate with Mayer's reagent [Potassium mercuric iodide solution].

Wagner's test: A fraction of extract was treated with 3-5drops of Wagner's reagent and observed for the formation of reddish brown precipitate (or colouration).

Tannic acid test: Alkaloids give buff colour precipitate with 10% Tannic acid solution.

Carbohydrates

Molisch's test: Few drops of Molisch's reagent were added to 2ml portion of the various extracts. This was followed by addition of 2ml of conc. H_2SO_4down the side of the test tube. The mixture was then allowed to stand for two-three minutes. Formation of a red or dull violet colour at the interphase of the two layers was a positive test.

Flavonoids

Alkaline reagent test: 2ml of extracts was treated with few drops of 20% sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid, indicates the presence of flavonoids.

Phenols

Ferric chloride test: A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour

Saponins

Foam test: To 2mls of extract was added 6ml of water in a test tube. The mixture was shaken vigorously and observed for the formation of persistent foam that confirms the presence of saponins.

Sterols

Liebermann-Burchard test: 1ml of extract was treated with drops of chloroform, acetic anhydride and conc. H2SO4 and observed for the formation of dark pink or red colour.

Terpenoids

Salkowki's test: 1ml of chloroform was added to 2ml of each extract followed by a few drops of concentrated sulphuric acid. A reddish brown precipitate produced immediately indicated the presence of terpenoids.

Tannins

Braymer's test: 2mls of extract was treated with 10% alcoholic ferric chloride solution and observed for formation of blue or greenish colour solution.

Quinones

A small amount of extract was treated with concentrated HCL and observed for the formation of yellow precipitate (or colouration).

°C for colour development. Absorbance was then measured at 765nm using spectrophotometer.

2.3 Antibacterial Activity By Disc Plate Method

Antibacterial activity of different solvent extracts was determined by disc diffusion method on nutrient agar medium. Seeded broth containing test organism was inoculated on plates of solidified nutrient agar and spread uniformly. Filter paper discs were prepared with a diameter of 3mm. In every plate petroleum ether, hexane, methanolic and water extracts containing discs are placed. The treatments also included filter paper discs immersed in DMSO and a standard streptomycin. The plates were incubated for 24 h. at 37°C and zone of inhibition around the discs were measured in mm (millimeter).

For the antibacterial activity the following strains are used:

-Salmonella typhi[NCIM 2501]

-Staphylococcus aureus[NCIM 2654]

-Pseudomonas aeruginosa[NCIM 2036]

-Escherichia coli [NCIM 2065]

3. RESULTS AND DISCUSSION

3.1 Qualitative Tests For Phytochemicals:

Table 1: Phytochemical analysis of *Bauhinia variegata* (Bark)

Phytochemicals	Petroleum ether	Hexane	Methanol
Alkaloids	-ve	-ve	+ve
Tannins	-ve	-ve	+ve
Flavonoids	-ve	-ve	-ve
Phenol compounds	-ve	-ve	+ve
Quinones	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Terpenoids	-ve	-ve	+ve
Anthroquinoes	+ve	+ve	-ve

From the results, few phytochemicals were absent in most of the solvent extractions like flavonoids, terpenoids, phenol compounds. Few phytochemicals showed presence in most of the solvent extractions like quinones, steroids.

Table 2: Phytochemical analysis of *Triticum aestivum*

Phytochemicals	Petroleum ether	Hexane	Methanol
Alkaloids	-ve	-ve	+ve
Tannins	-ve	-ve	+ve
Flavonoids	-ve	-ve	-ve
Phenol compounds	-ve	-ve	-ve
Quinones	+ve	+ve	+ve
Steroids	+ve	+ve	+ve
Terpenoids	-ve	-ve	-ve
Anthroquinones	+ve	+ve	-ve

From the result, few phytochemicals were absent in most of the solvent extractions like flavonoids, terpenoids, phenol compounds. Few phytochemicals showed presence in most of the solvent extractions like quinones, steroids.

3.2 Antibacterial activity of *Triticum aestivum*

The results were obtained by carrying out Disc Diffusion method on Nutrient Agar plate. The *Triticum aestivum* extracts from different solvents showed different range of zone of

inhibition. The major antibacterial activity was observed for gram positive bacteria. The maximum zone of inhibition for *S. typhi* was (hexane-8mm), *S.aureus* (Petroleum ether-8mm), *P. aeruginosa* (Petroleum ether and hexane-7mm) and *E.coli* (Petroleum ether- 6mm) with standard (streptomycin 1mg/ml) having zone of 22mm.

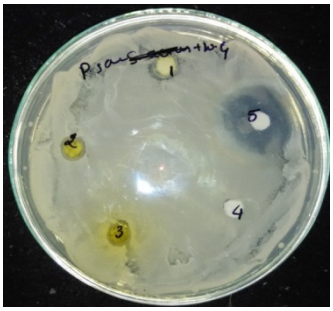

Fig. 1: Antibacterial activity of *Triticum aestivum* on *Pseudomonas aeruginosa*

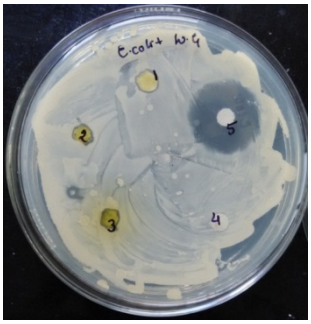

Fig. 2: Antibacterial activity of *Triticum aestivum* on *E. coli*

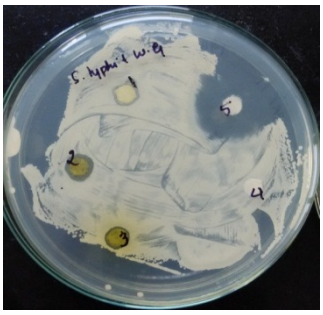

Fig. 3 :Antibacterial activity of *Triticum aestivum* on *Salmonella typhi*

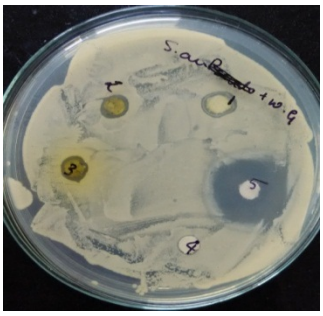

Fig. 4: Antibacterial activity of *Triticum aestivum* on *Staphylococcus aureus*

3.3 Antibacterial activity of *Bauhinia variegata*

The above results were obtained by carrying out Disc Diffusion method on Agar plate. The *Bauhinia variegata* bark extracts from different solvents showed different range of zone of inhibition. The major antibacterial activity was observed for gram positive bacteria. The maximum zone of inhibition for *S. typhi* was (Methanol-11mm), *S. aureus* (Petroleum ether, Methanol, Hexane-6mm), *P. aeruginosa* (Petroleum ether, Methanol, Hexane-7mm) and *E.coli* (Methanol-9mm) with standard (streptomycin 1mg/ml) having zone of 22mm.

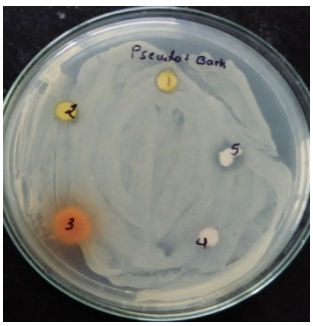

Fig. 5: Antibacterial activity *of Bauhinia variegata* on *Pseudomonas aeruginosa*

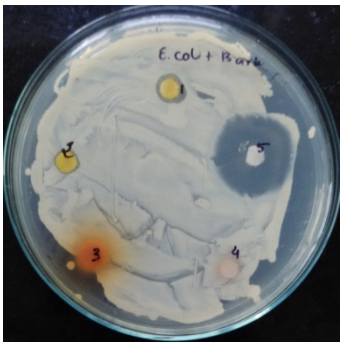

Fig. 6: Antibacterial activity of *Bauhinia variegata* on *E. coli*

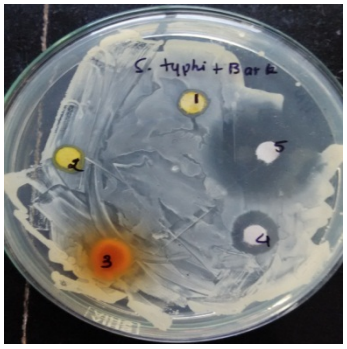

Fig. 7: Antibacterial activity of *Bauhinia variegata* on *Salmonella typhi*

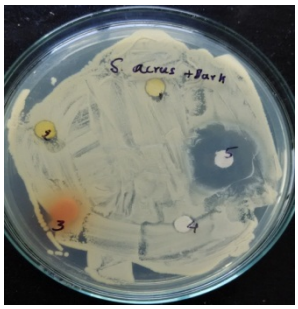

Fig. 8: Antibacterial activity of *Bauhinia variegata* on *Staphylococcus aureus*

3.4 Antibacterial activity of both *Bauhinia variegata* and *Triticum aestivum*

The following results were obtained by carrying out Disc Diffusion method on Agar plate. The mixture of both plant extracts from different solvents showed different range of zone of inhibition. The major antibacterial activity was observed for gram positive and gram negative bacteria.

The maximum zone of inhibition was observed for the combined effect of the plant extracts than the individual plant extracts. The maximum zone of inhibition for *S. typhi* was (Hexan-10mm), *S. aureus* (Methanol, Hexane-10mm), *P. aeruginosa* (Methanol-12mm) and *E.coli* (Methanol-9mm) with standard (streptomycin 1mg/ml) having zone of 25mm.

Fig. 9: Combined antibacterial activity of *Triticum aestivum and Bauhinia variegata* on *Pseudomonas aeruginosa*

Fig. 10: Combined antibacterial activity of *Triticum aestivum and Bauhinia variegata* on *Escherichia coli*

Fig. 11: Combined antibacterial activity of *Triticum aestivum and Bauhinia variegata* on *Salmonella typhi*

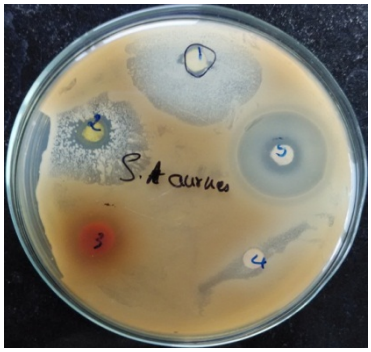

Fig. 12: Combined antibacterial activity of *Triticum aestivum and Bauhinia variegata* on *Staphalococcus aureus*

4. DISCUSSION

Antibacterial activities of various phytochemicals have been studied for their potential uses against infectious diseases. Emergence of multiple drug resistance in human pathogenic organisms has led to a search for new antimicrobial substances from alternative natural sources. Plants are known to produce certain chemicals which are naturally toxic to microorganisms especially against multidrug resistant bacteria. The present study revealed the presence of secondary metabolites such as terpenoids, phenolics, quinones, anthraquinones, sterols, tannins, and alkaloids in *B. variegata and Triticum aestivum* extracts (Tables 1 and 2). Some of the extracts derived from *B. variegata* and *Triticum aestivum* exhibited substantial antibacterial activities (Table 3 and 4). Some of the test bacteria (E.coli) showed resistance to the methanolic and hexane extracts of *T. aestivum* and the mixture of both the plant extracts. Gram negative bacteria are frequently reported to have developed multidrug resistance to many of the currently available antibiotics [12]. But it was surprisingly seen that *Pseudomonas aeruginosa, a* gram negative bacterium, was also inhibited by the extracts of both plants and showed larger zone of inhibition for the mixture. (120 mm; and zone of std=300mm). Degree of variability in the antibacterial activity could be attributed to the differential composition of phytochemicals present in extracts (Table 1

and 2). Plant extracts and essential oils may exhibit different modes of action against bacterial strains, such as interference with the phospholipids bilayer of the cell membrane which has as a consequence a permeability increase and loss of cellular constituents, damage of the enzymes involved in the production of cellular energy and synthesis of structural components, and destruction or inactivation of genetic material. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents (Kotzekidou *et al.,* 2008) (Fig. 1). The results of our work on phytochemical analysis varied from that of the work carried out by J. David Phillipson 2000 [1], Amita Mishra [3], Satyavati Rana [4], Dhale D.A. 2011 [5], S.K.Jash *et al* [10]. It is worthy of note that antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study [11,13]

5. CONCLUSION

The plant of *Triticum aestivum* and *Bauhinia variegata* is an indigenous herb which were chosen for this study. *T. aestivum* belongs to the family Poaceae and Caesalpiniaceae. The scanty availability of information on these plants facilitates the study on it since ages these plants are being used for its medicinal value. Various useful attempts have been made for different types of study of the plants. This attempt is made to study the different phytochemicals present and its quantification. Study was also carried out for antibacterial action of the plant extract on gram positive and gram negative strains. WE have made a new attempt to study the antibacterial activity of the mixture and it was found that the antibacterial activity of the mixture of both the plant extracts was more significant. Hence, these properties can be studied further and drugs can be designed so that the plants can be used effectively to its full potential.

6. ACKNOWLEDGEMENT

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