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Evaluation of antimicrobial activity of *Tinospora cordifolia* and *Hymenocallis Littoralis* medicinal plants by using different solvents extract

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Abstract - ***The present study reports that the screening of antimicrobial activity of Tinospora cordifolia and Hymenocallis littoralis, medicinal plant's extracts with various different plant parts such as bulb, root, leaves and stem against the opportunistic organisms such as Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium and fungi Candida albicans using agar well diffusion method and Minimum inhibitory concentration (MIC). Antimicrobial efficiency of Tinospora cordifolia and Hymenocallis littoralis, medicinal plants were examined using Ethanol, Methanol, Chloroform, Dichloromethane, ethyl acetate as solvents a nd found that all the plants parts showed significant activity against all pathogens. The best MIC (zone of inhibition in mm) results were found to be in case of Staphylococcus aureus by using methanol stem crude extract of Tinospora Cordifolia . In case of Hymenocallis littoralis the best MIC (zone of inhibition in mm) results were found to be in case E.coli by using methanol bulb crude extract . The Spectrum of activity observed in the present study may be indicative of the present study methanol extracts of these plants could be a possible source to obtain new and effective herbal medicines to treat different diseases or disorders.***

Key Words: ***Tinospora cordifolia, Hymenocallis littoralis, Antimicrobial, MIC, Agar Well Diffusion.***

1. INTRODUCTION

The discovery and production of antibiotics are some of the maximum powerful and success achievements of present-day science and generation for the control of infectious diseases. But, the rate of resistance of pathogenic microorganisms to conventionally used Antimicrobial agents is increasing at an alarming. Isolation of microbial agents much less liable to natural antibiotics and restoration of resistant isolates all through antibacterial therapy is growing around the world (Cohen 2002). Further to this problem antibiotics are from time to time related to effects on the host, which consist of hypersensitivity, depletion of the beneficial intestine and mucosal microorganisms, immunosuppression, and allergies. The variety of multi-drug resistant microbial traces and the arrival of lines with decreased susceptibility to antibiotics are continuously increasing. This boom has been attributed to the indiscriminate use of wide-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Ng 1994; Dean and Burchard, 1996; Gonzalez et al., 1996). Examples encompass methicillin-resistant staphylococci, pneumococci proof against penicillin and macrolides, vancomycin-resistant Enterococci as well as multi-drug resistant gram-negative organisms (Norrby et al. 2005). There is an urgent need to control antimicrobial resistance by improved antibiotic usage and reduction of hospital cross-infection , but, the development of new antibiotics have to be continued as they may be of primary significance to hold the effectiveness of antimicrobial remedy (van der Waaij and Nord 2000; Marchese and Shito 2001). The potential for development of new antimicrobials from higher plant life seems rewarding as it will cause the development of a phytomedicine to act against microbes; as a result, vegetation is one of the bedrocks for cutting-edge remedy to gain new standards (Evans et al. 2002). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. Further persevered exploration of plant derived antimicrobials is needed nowadays (Hussain and Gorsi 2004). Historically, the plant has supplied a source of suggestion for novel drug compounds, as plant derived drugs have made massive contributions to human health and well-being (Iwu et al. 1999). Medicinal plants represent a powerful source of each traditional and current drug treatments. Natural remedy has been proven to have genuine utility, and about 80% of rural populace relies upon on it as primary fitness care (Farnsworth et al. 1985; Akinyemi et al. 2005). Through the years, the WHO encourages traditional medicinal drug for you to identifying and exploiting elements that provide secure and effective treatments for illnesses of

both microbial and non-microbial origins (WHO 1978). In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population .

2. MATERIALS AND METHODS

2.1 Plant materials

Root,Stem, leaves of *Tinospora cordifolia* and Root,leaves bulb, stem of *Hymenocallis littoralis* used in this study were collected from Bundelkhand region.

2.2 Plant identification

Collected plants are identified by by Mr .Rajesh Mudaiya from Central council for research in aryuvedic sciences ,Jhansi

2.3 Drying of plant sample

After the collection of the sample, it needs to be dried to make the sample extract. In general, the plant material should be dried at temperature below 300ºC to avoid the decomposition of thermolabile compounds. Plant drying under the sun be very effective, but the disadvantage is sometimes water molecules are soaked by the sample, and hence microbial growth can affect the phytochemical study. The plant were dried in the sunlight thus chemical decomposition cannot take place.

2.4 Grinding of dried sample

A small quantity of plant material may be milled using grinder or blender. But if the sample is in excessive quantity then it is less complicated to get a powdered sample by means of grinding from a spice mill. Grinding improves the performance of extraction through the increased surface area. It also decreases the quantity of solvent required for the extraction. The dried samples were a coarse powder with a mechanical grinder (Blender), and powdered samples had been kept in clean closed glass packing containers pending extraction. At the time off of grinding of the sample, the grinder cleaned to keep away from contamination with any remnant of earlier ground material.

2.5 Preparation of extract for antimicrobial assay

Hymenocallis littoralis plant parts (Bulb, Stem, leaves, and root) were cautiously cut and washed with running tap water and then with clean, sterile water to remove dirt before the drying process. Every plant parts were cut into small pieces and dried at 48 hours at 45°C for to eliminate the moisture content. Finally, explants was ground using mortar and pestle into fine powder sample.

2.6 Preparation of extract for antimicrobial assay

Each plant parts Bulb, leaves and root of *Hymenocallis littoralis* and stem ,leaves,root of *Tinospora cordifolia* were carefully cut and washed with running tap water and then with clean sterile water to remove dirt prior to the drying process. Each of the plant parts were cut into small pieces and dried at 45°C for 48 hours to remove the moisture content. Finally, explants was ground using mortar and pestle into fine powder sample.

2.7 Solvent Extractions

The powdered plant materials have been extracted the usage of specific solvents Ethanol, Methanol, Chloroform, Dichloromethane, Ethyl acetate via maceration approach to gain the crude extracts. 1 gram of dried samples become ground the use of pestle and mortar, earlier than including 10 ml of methanol. Through filtered four layers of miracloth and centrifuged at 4700 rpm for 5min at 25°C. Supernatent become taken for similarly research or have been stored below refrigeration (-20°C) condition for further analysis.

2.8 Test Microorganisms. Escherichia coli , Salmonella typhimurium, Staphylococcus aureus, Candida albicans , Pseudomonas aeruginosa were used for screeing .

3 . AGAR WELL DIFFUSION ASSAY

Antimicrobial activities of different extracts were evaluated by the agar well diffusion method. Nutrient agar (NA) and Sabouraud's Dextrose Agar (SDA) plates were poured inoculated with 24 hrs and 48 hrs old broth culture of bacterias and fungi respectively under aseptic conditions. The plates were covered and allowed to cool. As soon as the agar was partly solidified, the plates were inverted and left for 2 hrs. Then, wells were made at the center of the plate by using a 6 mm cork borer that was sterilized with alcohol and flame. A stock solution of each plant parts extract was prepared at a concentration of 1 mg/10ml in methanol. About 100 µl of different concentrations of plant solvent extracts were added by sterile micropipettes into the wells and allowed to diffuse at room temperature for 2 hrs. The five solvents viz., ethanol, methanol, chloroform, dichloromethane, ethyl acetate at different volumes were used as control whereas Gentamycin and Caspo at same concentration with plant extract was used as the reference. The plates were incubated at 37°C for 18-24 hrs for bacterial pathogens and 28°C for 48 hours

fungal pathogens. The diameter of the inhibition zone (mm) was measured, the readings were taken in three different fixed directions, and the average values were recorded.

4. RESULT AND DISCUSSION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the natural therapies. In the present study, five solvents extracts viz Ethanol, Methanol, Chloroform, Dichloromethane, Ethyl acetate were taken for antimicrobial activity by agar well diffusion method where in comparision with other solvents, Methanol crude extract of Tinospora cordifolia showed best activity against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium and Candida albicans.

The best MIC (zone of inhibition in mm) results were found to be 20 mm in case of Staphylococcus aureus by using stem crude extract followed by the Escherichia coli (19mm), Salmonella typhimurium (19mm), Pseudomonas aeruginosa (17mm) and Candida albicans (19 mm). Stem crude extract were shown best activity against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa Salmonella typhimurium and Candida albican are followed by roots, leaves and bulbs crude extract of *Tinospora cordifolia* are given in Table.4.1

Five different Solvents samples and their crude extracts (Ethanol, Methanol, Chloroform, Dichloromethane, Ethyl acetate) of different parts such as Roots, leaves and bulbs from *Hymenocallis littoralis* were used to screen the antimicrobial activity, Meanwhile, five microbes were used to test all the samples, namely Gram positive bacteria (Staphylococcus aureus), Gram negative bacteria (Salmonella typhiimurium, Pseudomonas aeruginosa, Escherichia coli and fungal strain (Candida albicans). Overall, bulb crude extract showed pronounced inhibition of microbe's growth of the cultures compared to Roots, Leaves extract with different range on inhibition zone (Table.4.2). Most probably, the highest amount of secondary metabolite was presented such as an alkaloid in mature plant was the main reason for these results obtained.

Bulb extracts from the *H. littoralis* against Escherichia coli followed Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhiimurium, Candida albicans. produced highest zone of inhibition at 23 mm followed by 20 mm, 19 mm, 17mm and 19mm respectively, bulb crude extracts produced highest activity against above given bacterial and fungal strains in comparision with Roots, leaves accordingly.

The plant extracts showed a clear inhibition zone for all the tested samples. This findings show, H. littoralis plant extract is susceptible for bacteria and fungus.

Table .4.1 :Antimicrobial activity of Methanol crude extract of *Tinospora cordifolia*

Microorganisms	Zone of inhibition MM				
	Test samples				
Gram –ve and gram +ve	Root extract	stem extract	Leaves extract	Gentamycin 1mg /10 ml	Capso 1mg/ 10 ml
Staphylococcus Aureus	18	20	16	24	——
E.coli	17	19	16		
Pseudomons	15	17	13		
S.typhiimurium	16	19	15		
C.albicans	13	19	13	——	22

Table .4.2: Antimicrobial activity of Methanol crude extract of *Hymenocallis littoralis*.

Microorganisms	Zone of inhibition MM				
	Test samples				
Gram –ve and gram +ve	Root extract	Leaves extract	Bulb extract	Gentamycin 1mg /10 ml	Capso 1mg/ 10 ml
Staphylococcus Aureus	14	18	20	25	——
E.coli	16	21	23		
Pseudomons	15	18	19		
S.typhiimurium	14	16	17		
C.albicans	16	15	19	——	22

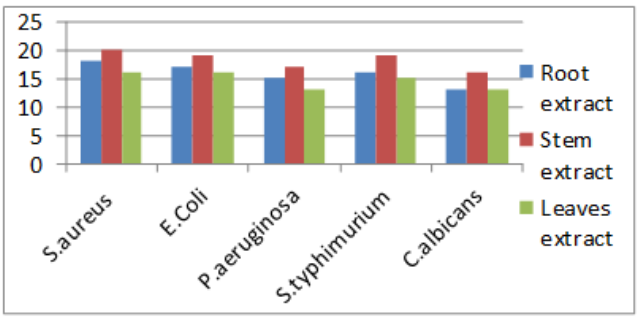

Fig. 4.1 : Well Agar Diffusion Assay of Methanol Extract of *Tinospora cordifolia*

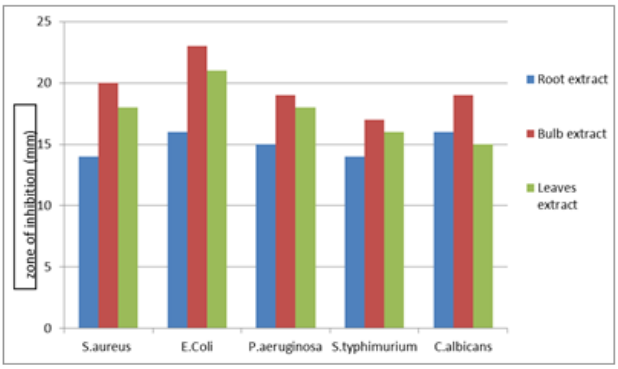

Fig.4.2:Well Agar Diffusion Assay of Methanol Extract of *Hymenocallis littoralis*

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