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SCREENING OF TRADITIONALLY USED *GLYCYRRHIZAGLABRA (MULETHI)* FOR POTENTIAL ANTIBACTERIAL ACTIVITY

Sharma A[1], Ojha A[2], Shamra P[3], Mishra R[4]

[1,2]Centre for Converging Technologies, University of Rajasthan, Jaipur, Rajasthan, India.

[3,4]R&D, Division, MRD LifeSciences Pvt. Ltd. Lucknow, UP, India.

***Corresponding Author: Pallavi Sharma, Email ID**: pallsharma91@gmail.com

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ABSTRACT

The *Glycyrrhiza glabra* (mulethi) are consumed throughout the country, antibacterial property of aqueous, polar and nonpolar extracts of this plant and tetracycline as positive control was checked against *Mycobacterium tuberculosis, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhimurium, Clostridium, Lactobacillus rhammanus, Lactobacillus acidophilus, Lactobacillus fermentum*and *Staphylococcus aureus* through agar well diffusion method. Polar and nonpolar extracts of *Glycyrrhiza glabra* shows antibacterial results except aqueous extract. In which the acetone extract has potential to inhibit the maximum growth of pathogens as compare to extract from other solvents. Antibacterial screening of analgesics were performed by making the combinations with the plant extracts in (1:1), (1:4) and (4:1). In which *Glycyrrhiza glabra* acetone extract with nimesulide combination enhanced the antibacterial properties of nimesulide after that the dosage of this modified drug was calculated by using minimum inhibitory concentration test. For the identification of the compounds present in respective plant phytochemical test were carried in which mainly alkaloids, terpenoids, flavonoids etc. were mainly present.

Key word: Phyto-compounds, Metabolites*, Glycyrrhiza glabra,* Analgesics, Antibacterial.

INTRODUCTION

Natural products arerealways good for health and plants as the sources of natural productsand have a broad diversity in the world **[1].**Plantcontainsphytochemicals like tannins, flavonoids, phenols, steroids, saponins, terpenoids which are good sources forthe treatment of infectious diseases **[2].** Now a day'suse of herbal products increasing fast due to low cost, highly effective and low chance to cause side effects **[3].**India and china contributed about 80% of total natural drugs production on the other hand developed countries like a united nation total contributed about 25% of total herbal drugs production **[4].** Infectious diseases were also increase very rapidly and harm lots of peoples every day in the world **[5].** Mostly the treatment of these infectious diseases were done by using the synthetic compounds, which is costly, moderate and good effective and also have the chance to cause side effects **[6].**Mostly microbes are responsible for the causing the infectious diseases to the humans, most of the microbes are classified in two parameters such as gram positive and gram negative **[7].**Many infections are caused by the gram negative bacteria throughout the body.

The infection sites are lungs, urinary tract, blood stream, nervous system, tissues which are soft in nature etc., these bacteria can also make the surgical wounds infected which can cause lots of infectious diseases **[8].** Whereas gram positive bacteria shows a main public health load, not in the terms of morbidity and humanity but also in terms of greater than before expenses on patient management and accomplishment of infection be in command of measures [9]. In preset era, the microbes are becoming resistant against drugs, the resistive nature or pathogenic microorganisms are increasing day by day **[10].** The plant *Glycyrrhiza glabra (mulethi), is* consumed throughout the country, the plant are having lots of properties such as antimicrobial, anti-stress, anti-diabetic, anti-inflammatory and much more **[11].** The roots of *Glycyrrhiza glabra*used throughout the world to cure cough since long times **[12].** It is an herb as flavor and also contains sweet flavor, which is added to candies and soft drinks. It is the important component of Ayurvedic and Allopathic system of medicines **[13].** It is one of the important herbal drugs which issued for the cure of skin disorders **[14].**

MATERIALS & METHODS

Test organisms

The bacterial cultures which are used for the tests are *Mycobacterium tuberculosis, Pseudomonas aeruginosa, Klebsiella pneumonia, Salmonella typhimurium, Clostridium, Lactobacillus rhammanus, Lactobacillus acidophilus, Lactobacillus fermentum and Staphylococcus aureus*available at the MRD LifesSciences, Pvt. Ltd. Lucknow. These bacterial cultures were maintained over here by sub-culturing it.

Extract preparation

The samples were collected from local Pan Shop, Gomti Nagar, Lucknow (UP) and then converted into powder. After that the samples were dipped in methanol, chloroform, acetone and petroleum ether and incubated for 24 – 48 hours at room temperature. The samples were filtered in the weighed bowl and incubated till the solvents evaporate from it. The remaining dried extracts were dissolved in DMSO and preserved for further use **[15].**

Antibacterial susceptibility test of the drug

The antimicrobial screening of the plant extracts were performed by using Agar well diffusion method against the test pathogens. The sterilized nutrient agar media was prepared and then the spreading of test pathogens was done. Then the plant extracts (drugs) were loaded to the well prepared over the plates and incubated at 37°C for 24 hours. The zone of inhibition was calculated **[16].**

Minimum inhibitory concentration test of drug

The minimum dose of drug which is responsible for the maximum inhibition of the test pathogens is done by Minimum Inhibitory Concentration (MIC) test. It is based on the broth dilution method, where the drugs are serially diluted in the sterilized broth and then the test pathogens were inoculated to it and incubated for 24 hours at 37°C. The growth OD was checked at 620 nm. Stoichiometric calculation is done to determine the MIC value of the extract for showing least value of optical density **[17].**

Phytochemical analysis

The tests were done for the detection of the presence of phyto-compounds **[18, 19, 20].**

Flavonoids:10% lead nitrate was prepared.1 ml of extract was added with 1 ml of lead nitrate. A yellow precipitate was observed that determined positive result for flavonoids.

Saponin:1ml of extract with 3 ml of distilled water was taken, mixed. Froth denotes positive result for saponin.

Tannin: Few drops of lead nitrate were added in 1 ml of extract.Precipitate was observed for positive result.

Steroids: 1 ml of extract was added with 2 ml of Chloroform and 2 ml of H_2SO_4.Reddish brown interface shows the positive result.

Terpenoids: 0.1ml of chloroform was added with 0.1 ml of extract.0.1ml of H_2SO_4.3Few drops of acetate shows Red color indicating positive result.

Carbohydrates: 0.1ml of extract was added with 0.1 ml of Fehling A.0.1 ml of Fehling B was added in the solution and boil for 5 minutes.A red precipitate indicates positive result.

RESULTS

Sample collection and extract preparation

The collected samples were then grinded and converted into powder. The aqueous, polar and nonpolar extracts were prepared and then used for tests.

Antibacterial susceptibility tests

Antibacterial tests of acetone, ethanol, chloroform and petroleum ether, propanol, distilled waterextracts against *S. aureus, P. aeruginosa, K.pneumoniae, M tuberculosis, L. rhammanus, L. acidophilus, L.fermentous, Clostridium,S. typhimurium.* Where the acetone extracts shows the best potential to inhibit the growth of respective pathogens by showing maximum zone of inhibition.

Antibacterial sensitivity test of solvents against pathogens

The solvents alone were not able to inhibit the growth ofbacterial pathogens.

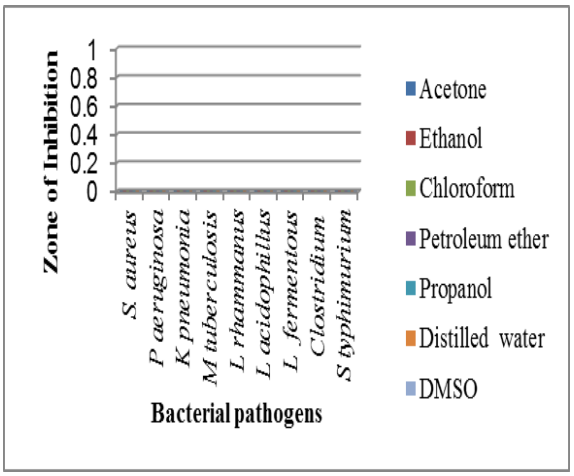

Figure1: Antibacterial effect of solvents towards the bacterial pathogens.

Antibacterial sensitivity test of analgesics against pathogens

These medicines were not good enough to show results for inhibiting the microbial growth.

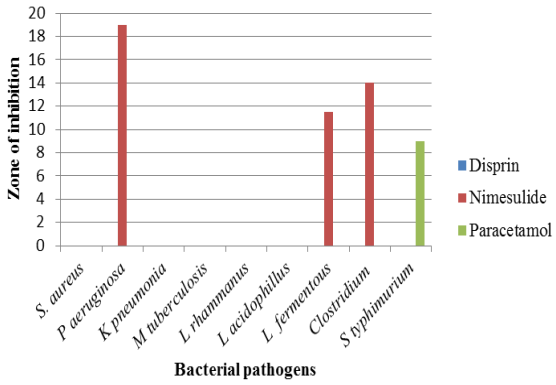

Figure 2: Antibacterial sensitivity test of analgesics against bacterial pathogens.

Antibacterial sensitivity test of *Glycyrrhiza glabra* extracts against pathogens

The polar, nonpolar and aqueous extracts of *Glycyrrhizaglabra* shows the better antibacterial properties against the pathogens but the acetone extracts have the potential for maximum inhibition of growth of pathogens as compare to other extracts.

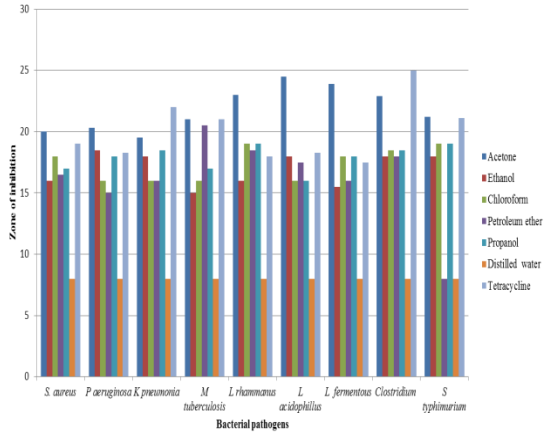

Figure 3: Antibacterial sensitivity test of acetone, ethanol, chloroform, petroleum ether, propanol, distilled water extracts of *Glycyrrhizaglabra* against bacterial pathogens.

3.2.4. Antibacterial sensitivity test of *Glycyrrhizaglabra*acetone extract with the combination of analgesics:

The combination of the *Glycyrrhizaglabra*acetone extract was prepared with the three different analgesics such as disprin, paracetamol and nimesulide in (1:1), (1:4) and (4:1) ratios for enhancing the properties of medicines.

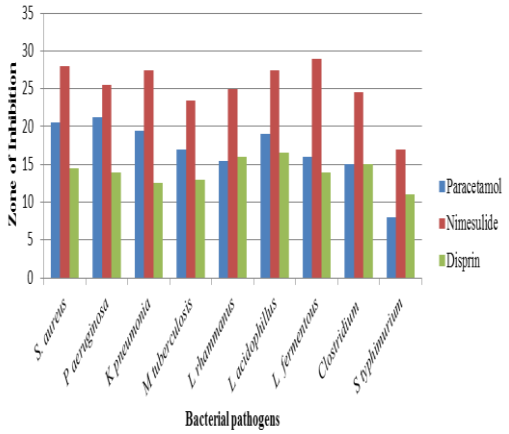

Figure 4: antibacterial sensitivity test of *Glycyrrhiza glabra*a cetone extract with the combination of paracetamol, nimesulide and disprin in 1:1 ratio against bacterial pathogens.

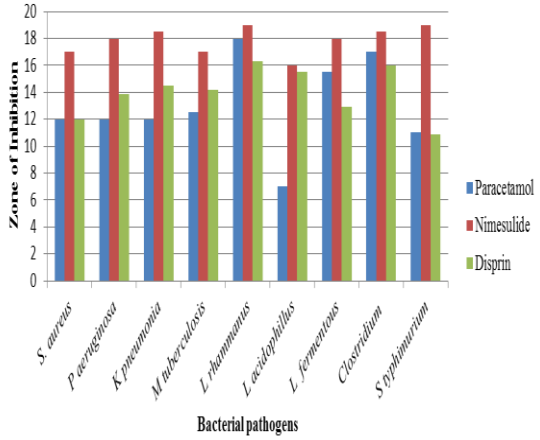

Figure 5: Antibacterial sensitivity test of *Glycyrrhizaglabra*acetone extract with the combination of paracetamol, nimesulide and disprin in 1:4 ratio against bacterial pathogens.

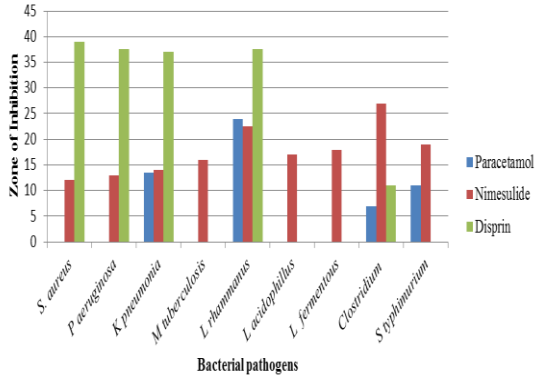

Figure 6: antibacterial sensitivity test of *Glycyrrhiza glabra* acetone extract with the combination of paracetamol, nimesulide and disprin in 4:1 ratio against bacterial pathogens.

Minimum inhibitory concentration test

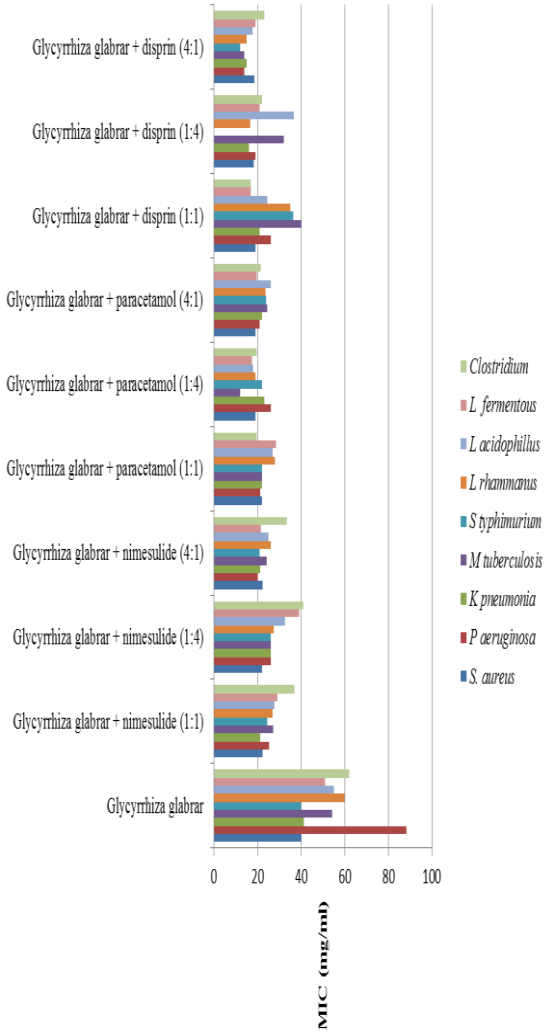

Figure 7:MIC values of the *Glycyrrhiza glabra* acetone extracts and with the combination of paracetamol, nimesulide and disprin in different ratios against bacterial pathogens.

Phytochemical Screening

Phytochemical	Acetone extract
Steroids	Negative
Flavonoids	Positive
Terpenoids	Positive
Saponin	Negative
Catecholictanin test	Positive
Gallic tannin test	Negative
Alkaloids	Positive

DISCUSSION

The recent few years have climate a change with a development of the world and development advancement in technology we have increase no antibiotics. The infection or diseases which were untreatable some year back are new no liger a warning. New bacterial pathogens which are multiple drug resistance have come into alive. To action such a plot we eat loss of medicines or antibiotics through we show direct result and they get heal in immune system achievable time, but these antibiotics have some side effect on us. Extent use of such over dose antibiotics which were use to heal us today make us unwell today. Mother Nature have a blessed us with the expensive riches of some medicinal plants, without being affected by giving us flowers, fruit to eat and wood for the making useful fuel lower for the aesthetic beauty. All what creation gives us the way for natural medication and we need search to benefit of human being and entering to this final way we selected a medicinal plant *Glycyrrhiza glabra* (mulethi) that assign its antibacterial activity. The various types of polar, nonpolar and aqueous solvents were used for preparing the extracts, and then the antimicrobial properties was checked in which the acetone extract shows the best results among them by showing the maximum zone of inhibition. The three analgesic medicines paracetamol, nimesulide and disprin were used to check the antibacterial property which shows that these were not able to show the good property. For enhancing the properties the drug modification was done by making the various combinations of these drugs with *Glycyrrhiza glabra* (mulethi). For checking the presence of the secondary metabolites present in plant the qualitative phytochemical analysis was performed.

Conclusion

We come up to a point where we can state that *Glycyrrhiza glabra* (mulethi) plants are good source of antibacterial compound and

can give to be a good source of natural medicine. It was investigated that the combinations of *Glycyrrhiza glabra* with the analgesics showed potential antibacterial activity of these analgesics against human pathogens.

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