



A STUDY OF ANTIMICROBIAL ACTIVITY OF POLYHERBAL EXTRACT OF *TERMINALIA BELLIRICA* & *GLYCYRRHIZA GLABRA* COMPOSITE

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ABSTRACT

The human skin is a primary and first line defense mechanism which protects the whole-body system. It is the huge protective system plays an important role in the avoiding of skin infections. Mostly bacterial and fungi species cause severe infections in the skin. The major causative agents are *Staphylococcus* sp, *Pseudomonas* sp. They are considered as a highly pathogenic microorganism. Dermatophytosis is the common disease that is caused by a wide range of fungal species. Clothing is called as a second skin and its helps for a healthy and contagious free living. It not only carriers of microorganisms such as pathogenic bacteria, odour generating bacteria and mould fungi, but also excellent source for the bacterial growth. Textiles are natural polymers of vegetable origin. It is manufactured for human to protect their bodies from temperature, dust, sunlight, wind and external environment. Medical textiles are a very important sector, which plays a vital role in health of humanity. Nowadays the environmental pollution and population increases the development of new hygiene products. The consumers are demanding eco-friendly, microbe free and comfortable products.

KEYWORDS : Dermatophytosis, polymers, eco-friendly product

INTRODUCTION

Generally, the plant extracts or herbal extracts are traditionally used for healing of wounds, burn injuries, anti-fungal, anti-viral, anti-bacterial and anti-microbial activity against skin infections. Herbal extracts are less toxic, less irritant and biodegradable and eco-friendly. Many plants possess antibacterial properties so it has a tremendous medical application. Recent days people are now returning to the uses of natural products due to the health concerns and safety issues. In India, all over 45,000 of different plant species of these are 15,000- 20,000 are probably medicinal value contain herbs. Different types of plant extracts used as drugs in medicinal system such as Unani and Ayurveda (Joy *et al.*, 2001). The drugs are derived from the various plants parts such as flowers, leaves, stem, bark, root, seeds. In modern medicine, herbal plants are considered more to cure of many human diseases (Adegoke *et al.*, 2009). Medicinal plants are around 250,000 species of higher plants on earth, including more than 80,000 medicinal plants. India is one of the mega centers of biodiversity in the world with an account of over 45,000 plant species. India's biodiversity is unmatched due to the presence of 16 distinct agroclimatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes, habitats of specific species (Joy 1998). In India about 17000 plants have good medicinal value. However, about 8,000 species are used in traditional drug systems for their medicinal values. In India, medicines of plant origin have been used in traditional systems of village communities, tribes, Unani and Ayurveda since ancient times. Plant possess a wide range of secondary metabolites and that have antimicrobial properties. They contain high content of phenolic compounds and flavonoids used to treat age-related disease such as Alzheimer's disease, Parkinsonism, anxiety, and depression (Sasidharan *et al.*, 2011). The secondary metabolites are a large group of biologically active compounds and it is synthesized as plant defense mechanism against various microorganisms, insects, herbivores, and different extreme of environmental conditions and for UV protection. Many research is shown that secondary metabolites have high biological activity (Latteef, 2016). Plant derived substances have variety of phytochemicals like tannins, phenols owing to their versatile applications. For the eco-friendly antimicrobial finishing some plants are identified and screened. Their extracts are applied to cotton fabric. Herbal extracts of *Terminalia bellirica* and *glycyrrhiza glabra* was selected for this study and to analyse the Phytochemicals constituents, evaluate the antimicrobial activity and minimum inhibitory concentration of the extracts of the *Terminalia*

bellirica and *Glycyrrhiza glabra* composite.

MATERIALS AND METHODS

Collection Of Plant:

The fruit of *Terminalia bellirica* and *Glycyrrhiza glabra* were collected from the local market of Coimbatore district.

Extraction of herbal extract:

The fruit of *Terminalia bellirica* and *Glycyrrhiza glabra* was separately taken and fine powdered using a clean mixer grinder. Each plant powder of 20 grams were boiled on 60°C in 100ml methanol for 30 minutes in heating mantle. The extracts were filtered through whattsman No.1 filter paper and the filtrates were used for the further process.

Phytochemical Analysis:

Test for Tannins:

To 1ml of extract add 3ml of bromine water. The disappear of extract colour indicates the presence of Tannins.

Test for Flavonoids:

Formation of yellow colour when 1ml methanolic extract of plant sample was added with 1ml of sodium hydroxide indicates the presence of Flavonoids.

Test for Saponins:

About 1 ml of distilled water was added to the 1ml of extract and the suspension was shaken vigorously. The foam formation indicates the presence of Saponins.

Test for Steroids:

To 1ml of extract about 2ml of acetic acid and 2ml of concentrated sulphuric acid was added. The appearance of blue or green indicated the presence of Steroids.

Test for Terpenoids:

To 1ml of extract about 2ml of acetic acid and 2ml of concentrated sulphuric acid was added. Red, pink or violet indicates the presence of Terpenoids.

Test for Glycosides:

Extract of 1ml was treated with 1ml of acetic acid and 1ml of ferric chloride and 1ml of concentrated sulphuric acid was added. The blue-green formation indicates the presence of Glycosides.

Test for phenol compounds:

To 1ml of extract, 1ml of ferric chloride was added. Black or green formation indicates the presence of phenolic compounds.

Test for Alkaloids:

Formation of blue when 1ml of extract was added to 1ml of iodine solution indicates the presence of Alkaloids.

Test for Quinones:

To 1ml of extract, 1ml of hydrochloric acid was added. Green, red or pink colour indicates the presence of Quinones.

Test for Protein:

Formation of yellow when 1ml of nitric acid added to 1ml of extract indicates the presence of protein.

Composite preparation:

Mix equal volume of the two extracts and kept in a magnetic stirrer with hot plate at 60°C for 30 minutes to prepare the composite (Shivani et al. 2019).

Antimicrobial Activity:

Test Organisms:

The antimicrobial activity was evaluated by using isolated strains. The bacterial strains were maintained using nutrient agar medium and sub cultured regularly.

Well Diffusion Method:

The antimicrobial activity of *Terminalia bellirica* and *Glycyrrhiza glabra* extract was evaluated by using agar well diffusion method. The sufficient amount of nutrient agar was prepared and sterilized at 121°C for about 15minutes. Standardized inoculum suspension (0.1) of each bacterial and fungal strain (*S. aureus*, *E. faecalis*, *klebsiella pneumoniae*, *C. albicans*, *C. tropicalis*) was spread on nutrient agar with sterile swabs. Then the agar plates were punched with a sterile cork borer size of 6mm and 100µl of each extract and composite was added to the corresponding well using micropipette. The plates were incubated for 24 hours at 37°C. After the incubation zone of inhibition was measured(mm) (Dhanasekaran et al.,2005).

Minimum Inhibitory Concentration:

For each organism 12 Eppendorf tubes were taken. In each tube 100µl of nutrient broth was added. To the first tube 100µl of extract is added and Serially diluted up to the last tube. From the last tube 100µl was discarded. After this 50µl of resazurin was added to the all tubes. Then 10µl of culture was added to each tube. The tubes were incubated at 37°C for 18-24 hours. The colour change was then assessed visually. Any colour changes from purple to pink or colourless were recorded as positive. The lowest concentration at which colour change occurred was taken as the MIC value. The average of three values was calculated and that was the MIC for the test material and bacterial strain transferred aseptically into the respective conical flask and the incubated at 37°C for 24 hours. After incubation, the growth is observed. The broth was discarded and washed with sterile distilled water twice and that was plated on nutrient agar and it was again incubated at 37°C for 24 hours (Eva Pinho et al.,2014)

RESULT AND DISCUSSION



Root of liquorice fruit of *T. bellirica* Picture shows the image of fruit *Terminalia bellirica* and root of *Glycyrrhiza glabra*. Liquorice root has been used worldwide as a medicine and as an industrial aroma for over 4000 years. *T. bellirica* is found commonly in India and it grows wild at an elevation of up to 2000m in variety of ecologies.

It has a beneficial pharamological application so in this study it was used for check the antimicrobial activity against *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Candida albicans*, *Candida tropicalis*)

Phytochemical Analysis Of Fruit And Root Extract Of Terminalia Bellirica And Glycyrrhiza Glabra:

The screening of phytochemical analysis of fruit *Terminalia bellirica* indicates the presence of flavonoid, saponins, phenols, proteins. Absence of tannins, steroids, terpenoids, glycosides, alkaloids, Quinones. The results are shown in table 1.

In the root *Glycyrrhiza glabra* indicates the presence of saponins, terpenoids, phenols. Absence of tannins, flavonoids, steroids, glycosides, alkaloids, Quinones, proteins.

The phytochemical analysis revealed that the presence of phytochemical components are active medicinal components. As a result, the samples are very rich in flavonoids, saponnins, phenols and protein. The methanolic extract justifies the presence of saponins and flavonoids in it. (Shivani et al. 2019) Phytochemical analysis of fruit and root extract:

Table 1: Antimicrobial Activity Of Fruit And Root Extract:

Phytochemical compounds	Fruit	Root
Tannins	-	-
Flavonoids	+	-
Saponins	+	+
Steroids	-	-
Terpenoids	-	+
Glycosides	-	-
Phenols	+	+
Alkaloids	-	-
Quinones	-	-
Protein	+	-

The agar well diffusion method was used to test antimicrobial activity of the extracts against skin infection causing bacteria and fungi. It is highly used to evaluate of the effect of plant extracts or any other antimicrobial activity on disease causing pathogens. The result of antibacterial activity of fruit and root are shown in table 2(Dhanasekaran et al.,2005).



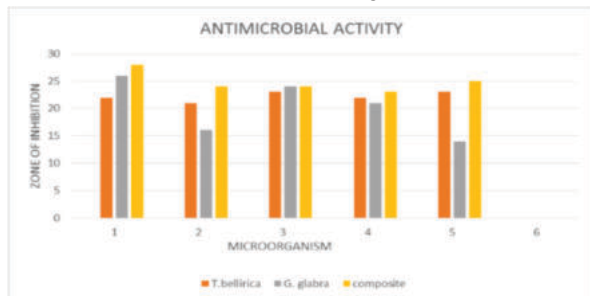


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Figure 6: Antimicrobial Activity of Individual Extract and Composite

The picture shows the antimicrobial activity of plant extract and composite against skin infection causing pathogens.

The zone of inhibition for composite is higher compared to the individual extract of *T. bellirica* and *G. glabra*.



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