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A COMPARATIVE STUDY OF ANTI-BACTERIAL AND ANTI-ULCEROGENIC ACTIVITY AND PHYTOCHEMICAL SCREENING OF ETHANOLIC EXTRACT OF GLYCYRRHIZA GLABRA(LICORICE) AND QUERCUS INFECTORIA (GALL NUT)

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Abstract

Medicinal plants have a great contribution to mankind by combating multiple health-related issues. In the traditional system, these remedies have the richest bioresource such as phenol, micro, and macronutrient. They can act as nutraceuticals, food supplements, and pharmaceutical intermediates. The present study deals with the Anti-bacterial and Anti-ulcerogenic activity of *Glycyrrhiza glabra* (licorice) and *Quercus infectoria*(nut gall). The medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents The ethanolic extract of *G.glabra* shows the presence of the following phytochemical compounds flavonoids, tannins proteins, and the *Quercus infectoria* show the presence of phytochemical compounds tannins, flavonoids, phenols. Antibacterial agents can be bactericidal killing their growth. They work by killing or inhibiting the growth of bacteria. Mouth ulcers are very common, occurring in association with many

diseases and by many different mechanisms, but usually, there is no serious underlying cause. The phytochemical compounds such as tannins and phenols are responsible for the Antibacterial and Anti ulcerogenic activity. From the present study, the ethanolic extract of the licorice and gall nut shows the antibacterial and Anti-ulcerogenic activity may be due to the presence of the phytochemical compounds like tannins, and flavonoids in licorice and the phytochemicals like proteins, flavonoids, phenol are responsible in nut gall.

KEYWORDS: *Glycyrrhiza glabra, Quercus infectoria,* phytochemical, antibacterial, Anti-ulcerogenic, tannins, flavonoids

Introduction

Siddha system of medicine is an ancient system of medicine prevalent in South India. Siddha treatment is aimed at restoring balance to the mind-body system. There is an equal emphasis on the body, mind, and spirit, and strives to restore the innate harmony of the individual. It is one of the earliest traditional medicine systems in the world which treats not only the body but also the mind and soul (kuppusamy Muthaliar *et al.,2009)*

Medicinal Plants

Due to the increasing awareness among people towards natural products, natural medicine is attracting more attention than allopathic systems. Moreover, this system of medicine is effective and less toxic without side effects (Malviya R et al., 2012).

The whole plant or its different parts may be valued for its therapeutic, medicinal, and aromatic qualities. Medicinal plants are cost-efficient and more accessible to most of the population in the world. Thus, there is a need to encourage the use of medicinal plants as potential sources of new drugs. There has been a highly increased interest in herbal remedies in several parts of the world (Daniyan SY et al., 2008).

They can act as nutraceuticals, food supplements, pharmaceutical intermediates, etc (Lalitha N 2013) An herbal-based formulation improves the quality of human life through its potent natural compounds. They provide a remedy for various chronic diseases and metabolic disorders which are multifactorial and therapeutic interventions (Rajani M et al) have a long history of beneficial plant materials that have provided the models for 25-50 drugs. Plants are the major source of natural secondary metabolites, which besides being an essential requirement for the plants to adapt to the environment also act as an active component by providing a range of potential health-beneficiary activities.

Natural products are potential sources because of their multiple therapeutic properties provided by their bioactive components. Moreover, in combination, the essential pharmacological properties are more effective. Phytochemicals are responsible for the medicinal properties of plant extracts and their probable mode of action has been keenly searched for a long time (Srivastava et al., 2014). The antioxidant property of plant extracts can prevent DNA damage from the carcinogens, if not it may, in turn, disturb the cell function. The therapeutic value

of plant extracts is chiefly from alkaloids and polyphenols; thus, they are much more concentrated than other secondary metabolites available in the plants.

Morphology

Quercus infectoria

The Quercus genus is an evergreen or deciduous tree, belonging to the Fagaceae family. *Quercus infectoria* (Family: Fagaceae) commonly known as gall oak, is a small shrub found in Greece, Asia Minor, and Iran. It is a small tree or shrub growing to 4 to 6 feet tall, crooked, with smooth and bright leaves, long and narrow, scaly and downy. It is also used as an ingredient in Siddha preparations.(Karioti. A, et al., 2009).

Fig:*Quercus infectoria*

Taxonomy of *Quercus infectoria*

Kingdom: Plantae

Sub kingdom: Viridiplantae

Division : Tracheophyta

Subdivision: Spermatophytina

Class :Magnoliopsida

Sub class :Hamamelididae

Super order : Rosanae

Order : Fagales

Family : Fagaceae

Genus : Quercus

Species : *Quercus infectoria*

Description

Quercus infectoria is a small tree native to Greece and Asia Minor, with one to two meters (four to six feet) in height. The stems are crooked, shrubby looking with smooth and bright-green leaves borne on Shon petioles of 3 to 4 cm (1 to 15 inches) long. Sting the oak tree and depositing their larvae the chemical reaction causes an abnormality in the oak tree causing hard balls to be formed. They are corrugated in appearance (Muhamad Z et al., 1994)

The plant *Quercus infectoria* Olivier (Family-Fagaceae) grows as a shrub or small tree, diclinous and monoecious. It is about 2.5 m in height with many spreading branches, Bark is slightly greyish. Leaves are rigid and glabrescent with spinous teeth measuring 4-6 cm in length. They are alternate, short petiolate, elongate, sinuate, roughly thorny-tipped serrate.

Glycyrrhiza glabra

Glycyrrhiza glabra is one of the most popular medicinal plants belonging to the Fabaceae family (also known as Leguminosae), and its members are now commonly used as feed and food. The genus Glycyrrhiza is derived from the Greek words glykos (sweet) and rhiza (root).

Taxonomy

Kingdom: Plantae

Subkingdom: Viridiplantae

Division: Tracheophyta

Subdivision: Spermatophytina

Class : Magnoliopsida

Super order:Rosanne

Order: Fabales

Family: Fabaceae

Genus: Glycyrrhiza

Species: *Glycyrrhiza glabra*

Description

G. glabra is a typical herbaceous perennial, growing to 1 m in height, presenting pinnate leaves with a length of 7 to 15 cm. The flowers are purple to pale whitish blue, being arranged in a hermaphrodite inflorescence, whereas the fruit is an oblong legume with 2 to 3 cm of length and containing several seeds.

Fig:Licorice

Phytochemical

Phytochemistry is the chemistry of plants or chemical constituents of plants. Phytochemistry is understood in pharmacy as the chemistry of natural products used as drugs or of drug plants with an emphasis on biochemistry. The constituents are therapeutically active or inactive. The inactive constituents are structural constituents of the plants like starch, sugars, or proteins. The inactive constituents have however pharmaceutical uses (Harbrone et al., 1999). Medicinal plants are useful for healing as well as for curing human diseases because of the presence of phytochemical constituents.

Antibacterial activity

Klebsiella are gram-negative, non sporing non-motile bacilli that grow well on ordinary media and produce pink mucoid colonies on Mac Conkey's agar. They are usually found in the intestinal tract of humans and animals or free-living in soil, water, and plants. Pseudomonas is rod-shaped, slender (0.5 to 0.8 µm by 1.5 to 3.0 µm) Gram-negative organism, motile by polar flagella, and sometimes more than two flagella may be present. Pseudomonas is a strict (obligate) aerobe, but sometimes it can grow anaerobically if nitrates (NO3 act as respiratory electron acceptor) are present in the medium. Pseudomonas can grow at a wide range of temperatures. Pseudomonas produces large, opaque, flat colonies with irregular margins and distinctively fruity odour colonies.

ANTI ULCEROGENIC ACTIVITY

A mouth ulcer is an ulcer that occurs on the mucous membrane of the oral cavity. Mouth ulcers are very common, occurring in association with many diseases and by many different mechanisms, but usually, there is no serious underlying cause. Rarely, a mouth ulcer that does not heal may be a sign of oral cancer. Once formed, an ulcer may be maintained by inflammation and/or secondary infection. An ulcer is a tissue defect that has penetrated the epithelial-connective tissue border, with its base at a deep level in the submucosa, or even within the muscle or periosteum. An ulcer is a deeper breach of epithelium compared to erosion or excoriation and involves damage to both epithelium and lamina propria. Pathologically, the mouth represents a transition between the gastrointestinal tract and the skin, meaning that many gastrointestinal and cutaneous conditions can involve the mouth. Some conditions usually associated with the whole gastrointestinal tract may present only in the mouth, e.g., orofacial granulomatosis/oral Crohn's disease.

Material and Methods

Sample collection

Quercus infectoria commonly called Gall nut- was purchased from the local country drug market in pattalamchennai. The samples were washed well with running tap water followed by distilled water, washed leaves were blotted on the blotting paper, shade dried at room temperature and the samples were ground into fine powder using a mixer grinder. The root of *Glycyrrhiza galbra* commonly known as *licorice* was purchased from the local country drug in Pattalam market Chennai. The sample was washed well with running tap water followed by distilled water, washed leaves were blotted on the blotting paper, shade dried at room temperature and the samples were ground into fine powder using a mixer grinder.

Fig: Gall nut

Fig : licorice

Preparation of sample extraction

250 grams of powdered samples were packed in a soxhlet apparatus and extract was collected. The condensate was collected and the solvent (ethanol) was evaporated using a rotary evaporator at 45°C and stored in room temperature until further use.

Soxhlet Extract

Soxhlet extraction was performed in a soxhlet apparatus. Exhaustive extraction with solvent (ethanol) was performed using 25g roots cut into small pieces, wrapped in filter paper, and impregnated with solvent. Extraction was performed with 500ml of solvent. The extract was then filtered through Whatmann No 1 filter paper. The result extraction yield was Collected for further experiment.

Preparation of Sample A

Sample A- ***Glycyrrhizagalbra***

Sample B - ***Quercus infectoria***

100mg/ml of samples A and B were weighed. These samples were dissolved in 1 ml of sterile distilled water and mixed well. From this different concentrations of samples A and B (25, 50, 75, and 100 µl samples) were loaded into the muller hinton agar plates. The sterile distilled water used as a negative control and respective antibiotics used as a positive control.

Fig: Showing powdered sample of A and B

(*Glycyrrhiza galbra*and *Quercus infectoria)*

PHYTOCHEMICAL ANALYSIS OF EHANOLIC EXTRACT OF SAMPLE

The phytochemical screening of ethanolic extract was done to detect the presence of following biomoleculesphenol (ferric chloride test)tannins (lead acetate test)flavonoids(Shinoda test)triterpenoids (Noller's test),proteins (Biuret test),glycosides,Quinones,Saponins, Coumarin reducing sugar (fehling's test)by standard qualitative phytochemical procedures

PREPARATION OF BACTERIAL SUSPENSION

Preparation of Muller hinton broth medium

Nutrient broth medium was purchased from Himedia Laboratories, Mumbai, India.

1.3 g of medium was dissolved in 100 ml of distilled water and sterilized by autoclaving at 15 lbs pressure (121 °C) for 15 minutes. The medium contained Beef extract, 2.0 ; Acid hydrolysate of casein, 17.5 ; Starch, 1.5 ; Agar, 17.0 per litre at pH 7.3±0.1 (25 °C).

Preparation of inoculums

Bacterial inoculums were prepared by transferring bacterial colonies from fresh culture tubes containing 5ml of Muller hinton broth (MHB) (from Hi-Media) and incubated for 24 hrs at 37°C. The tubes were shaken occasionally to aerate and promote the growth. The bacterial suspension was diluted to attain viable cell count 108 CFU/ml with a sterile physiological solution; its absorbance was adjusted at 580 nm using spectrophotometer.

RESULTS

Detection of antibacterial by zone of inhibition (ZI) assay:

Agar plates:

Mueller-Hinton agar was purchased from Himedia Laboratories, Mumbai, India for preparation of culture plates. 20 g of Mueller-Hinton agar was dissolved in 1000 ml distilled water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. About 15 ml of autoclaved agar medium (45oC) was aseptically poured into each Petri plate and allowed to cool and solidify. The occurrence of antibacterial activity in the crude and aqueous sample was analysed using “zone of inhibition (ZI) assay” with laboratory bacteria.

Mueller-Hinton agar was prepared as mentioned above. After solidification of agar in the plate, wells of about 5 mm in diameter were punched into the solid agar and 100 µl of the bacterial suspension were swabbed uniformly on the surface of MHA and the inoculum was allowed to dry for five minutes.

Then those wells were filled with different concentration of 25, 50, 75 µl and 100 µl of sample A and B 100 mg/ml on the respective plates. The plates were incubated for 24 h at 37 °C in inverted position. Positive (antibiotic disc) and negative controls (sterile distilled water) were also maintained.

Anti bacterial / Anti ulcerogenic activity of Sample A and Sample B (mg/ml concentration)

Sample	*Pseudomonasaeruginosa*			
Concentration	2.5	5.0	7.5	10
A	-	9	10mm	12mm
B	20 mm	24 mm	25 mm	25 mm
Ciprofloxacin	30 mm			

Sample	*Klebsiellapneumoniae*			
Concentration	2.5	5.0	7.5	10
A	-	-	-	-
B	15 mm	15mm	18 mm	20 mm
Tetracycline	18mm			

Sample A :*Glycyrrhiza glabra*
Sample B :*Quercus infectoria*
Antibiotics used : Ciprofloxacin for *Pseudomas aeruginosa*
Tetracycline for *Klebsiella pneumoniae*
No antibacterial activity

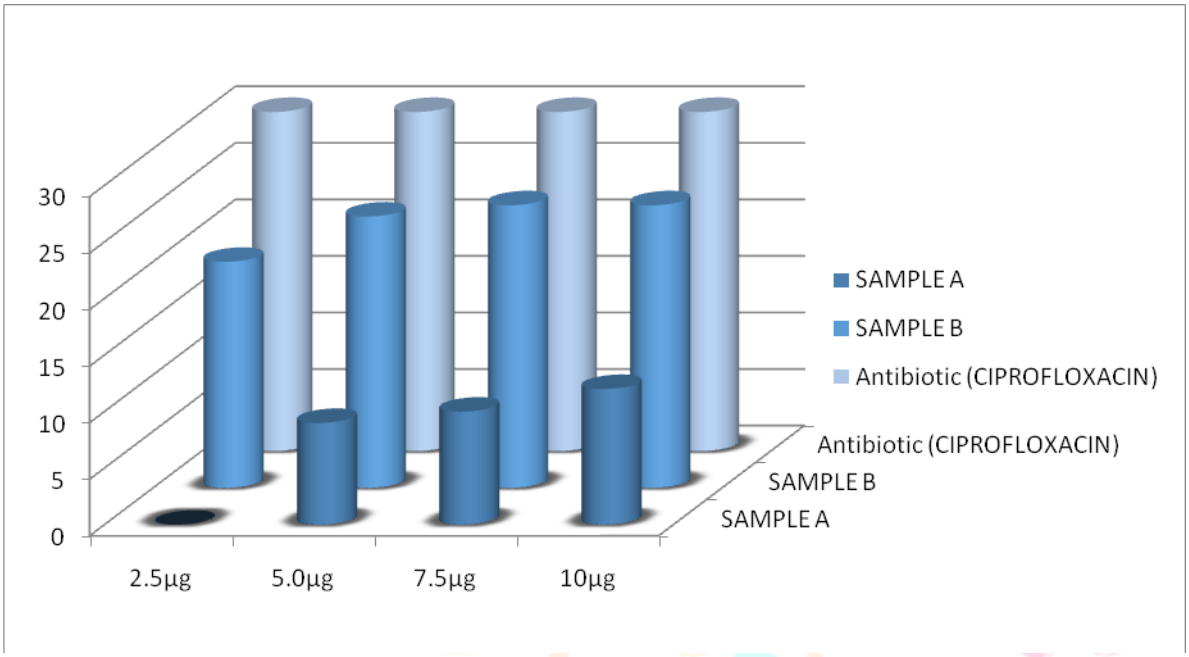

Fig: GRAPH SHOWING INHIBITORY EFFECT OF SAMPLE A&B ON PSEUDOMONAS AERUGONISA

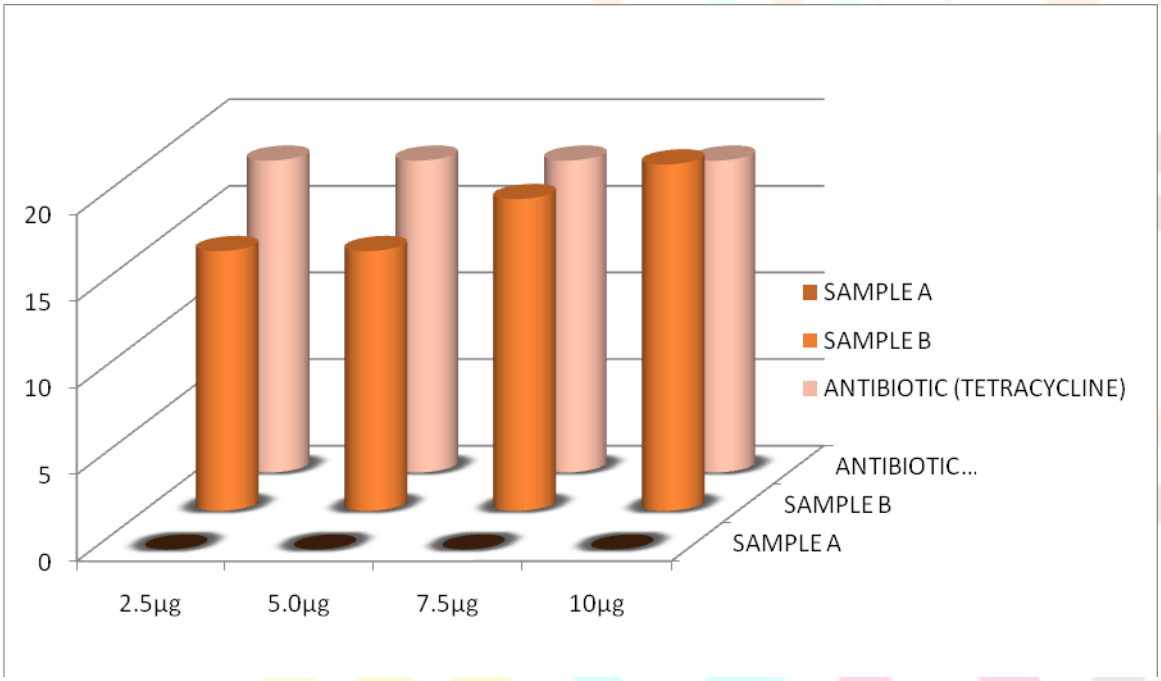

Fig: GRAPH SHOWING INHIBITORY EFFECT OF SAMPLE A&B ON KLEBSIELLA PNEUMONIAE

DISCUSSION

Medicinal plants have great contribution to mankind by combating with multiple health related issues. The present study deals with the Anti bacterial and Anti-ulcerogenic activity of *Glycyrrhiza glabra* (liquorice) and *Quercus infectoria*(nut gall). The ethanolic extract of *g.glabra* show the presence of following phytochemical compound flavonoids , tannins proteins etc and the *Quercus infectoria* show the presence of phytochemical compound tannins, flavonoids, phenol etc.,

The phytochemical compound such as tannins and phenols are responsible for the Anti- bacterial and anti ulcerogenic activity of the drug. The present study deals with the Anti bacterialand Anti-ulcerogenic activity of *Glycyrrhiza glabra* (liquorice) and *Quercus infectoria*(nut gall). The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents On the bacteria *Pseudomonas aeruginosa* on MH agar medium at the concentration range 2.5µg,5.0 µg,7.5 µg,10 µg show the maximum zone of inhibition of 12mm in diameter at 10ug and no effect was found with minimal concentration.At the 5.0 µg,7.5 µg concentration the zone of inhibition was 9mm and 10mm in diameter.

On the bacteria *Klebsiella pneumoniae* at the concentration range of 2.5,5.0,7.5,10 ug show the maximum zone of inhibition of 25mm in diameter both at 7.5 µg&10 µg and at 2.5 and 5.0 µg its 20mm and 24 mm in diameter.The ethanolic extract of *g.glabra* show the presence of following phytochemical compounds flavonoids,tannins proteins and the *Quercus infectoria* show the presence of phytochemical compounds tannins, flavonoids, phenols .Antibacterial agents can be bactericidal killing their growth. The phytochemical compound such as tannins and phenols are responsible for the Anti- bacterial and Anti ulcerogenic activity. From the present study,the ethanolic extract of the licorice and gall nut show the antibacterial and Anti-ulcerogenic activity may be due to the presence of the phytochemical compounds like tannis, flavonids in liquorice and the phytochemicals like protiens, flavonids , phenol are responsible in nut gall.

CONCLUSION

In traditional system, these remedies have a richest bioresource such as phenol, micro and macronutrient. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents .They can act as nutraceuticals, food supplement, pharmaceutical intermediates From the present study,the ethanolic extract of the licorice and gall nut show the antibacterial and Anti-ulcerogenic activity may be due to the presence of the phytochemical compounds like tannis, flavonids, in liquorice and the phytochemical like protiens, flavonids , phenol are responsible in nut gall.

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