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Research Article

Determination of Anti-microbial Property of Gycyrrhiza lepidota roots and Estimation of Phenolic compounds by using spectrophotometer

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ABSTRACT

Glycyrrhiza lepidota belonging to family fabaceae is well known for control of cough, chest pain, diarrhea, fever in children, stomachaches activity etc. Antioxidants are involves in disease controlling activity. Phenolic compounds are the major antioxidants present in the plan and involve in control of disease and protect the plants. FCM (Folin-Calciocaltio reagent) test was used to Estimate the Phenolic compounds in roots of Glycyrrhiza lepidota. Fc reagent was added to the Ethanolic extract and absorbance was measured at 720nm with spectrophotometer. And Phenolic compound was expressed as mg/gr of Gallic acid. About 23.92mg/100gr of Phenolic compounds are present in the ethanolic extract of roots of Glycyrrhiza lepidota. Antimicrobial property of methanolic etacts was studied by Disc plate method. Four Grm +ve and four Gram-ve bacteria are selected and discs containing methanolic extract were added in the plate. After incubation a clear zone formation reveals that plant roots of Glycyrrhiza lepidota has antimicrobial property. Among six it has inhibition activity against five bacterial sps. May be due to the presense of high amount of phenolic compound the plant roots has high antimicrobial activity.

Key words: Glycyrrhiza lepidota, Phenolic compounds, Spectrophotometer, anti-microbial activity, anti-oxidants.

INTRODUCTION

The currently accepted scientific name of wild licorice is Glycyrrhizin lapidate Push [1, 2, 3, 4]. It is a member of the Fabaceae family. It occurs in a variety of habitats but is most often found in prairie and other grassland communities or riparian areas. Wild licorice is a native, perennial, leguminous forb that grows from 1 to 4 feet (0.3-1.2 m) tall [3, 5, 6, and 7]. It may form colonies by adventitious shoots from roots and deep-seated rhizomes [1].

Scientific classification:

Kingdom : **Plantae**

Family : **Fabaceae**

Genus : **Glycyrrhiza**

Species : **G. lepidota**

The roots of wild licorice can be eaten, raw or cooked. They can be used as a flavoring in other foods as well and can also be chewed raw as a masticatory, making an excellent tooth cleaner and also very good for teething children. The root contains 6% glycyrrhizin, a substance that is 50 times sweeter than sugar. All parts of the plant are medicinal, but the roots are the most active part. Roots were used for toothache, fever and to strengthen the voice for singing by the Keres and Bannock Indians. A tea of the root was used to speed the delivery of the placenta after childbirth, it was also used to treat coughs, diarrhea, chest pains, fevers in children, stomach aches etc. Wild licorice can increase blood pressure as well. Glycyrrhizin, the sweet tasting compound is the acid ammonium salt of nitrogenous tri-basic acid, called glycyrrhizic acid. The potassium acid salt of this acid is very sweet. The free acid, prepared from the lead salt, forms a brown, gelatinous mass soluble in hot water and has a bittersweet taste and acid reaction. The root of wild licorice contains up to 6% Glycyrrhizin. Licorice can induce a hyper mineralocorticoid syndrome.

Antioxidants refer to the compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions and thus capable of counteracting the damaging effects of oxidation in animal tis- sues (Huang and others 2005). Antioxidants(8) are enzymes or other organic substances in foods that significantly decreases the adverse effects of reactive species, such as reactive oxygen and nitrogen species, on normal physiological function in humans. Phenolic compounds (9, 10, 11, and 12) found ubiquitously in plants, demonstrated potent antioxidant activity mainly due to their redox properties, which allow them to act as reducing agents, singlet oxygen quenchers, hydrogen donators, and chelating agents of metal ions (Rice-Evans and others 1996). They have wide range of biochemical and pharmacological actions such as antiviral, antimicrobial, and anti- inflammatory, and activities.

Material and Methods:

Chemicals: All Chemicals used were analytical grade and purchased from Merk Chemicals Pvt., Ltd. Mumbai, India. Methanol, FC reagent, Gallic acid Standard, Sodium carbonate solution.

Instruments: Soxhlet extractor, UV Spectrophotometer- Double Beam UV Spectrophotometer UV2301, Simple distillation apparatus, Laminar air flow, Auto cleave, Hot air oven.

Culture media: Nutrient agar medium (Composition: Peptone-, Agar-, Beef Extract-, Nacl-, Distilled Water-1000ml.)

Sample collections: Roots of Gycyrrhiza lepidota was collected from the Forest near Thirumala Hills, Thirupathi, Andhra Pradesh, India.

Preparation of Extracts: Roots of Gycyrrhiza lepidota were washed with Distilled Water and Shade Dried for 24 Hours and Powdered. The plant components were extracted in Soxhlet apparatus with Methanol solvent. The Methanolic extract was used for the estimation of anti-microbial activity and estimation of total Phenolic compounds.

Antimicrobial activity test:

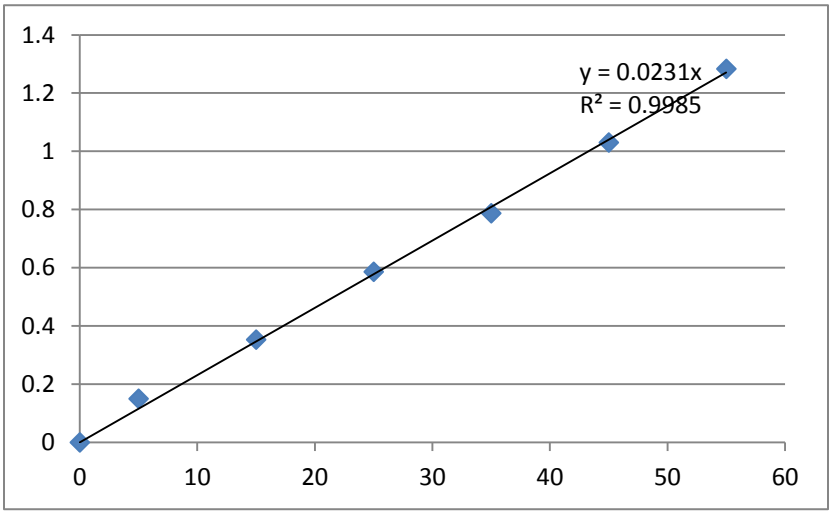
Nutrient Agar medium well-disc plate method: The nutrient agar medium disc plate method was used to screen the anti-microbial activity of all extracts of roots of glycyrrhiza lepidota. Seeded broth containing test organism was inoculated on plates of solidified nutrient agar and spread uniformly. Filter paper discs were prepared with a diameter of 3mm. In every plate methanolic extract containing discs are placed. Then all plates were incubated at 37^{o}C for 24hours. After the incubation period the mean diameter of the Zone of Inhibition In mm obtained around the well was measured which has been shown in the table.

Estimation of phenolic compounds by using spectrophotometry: Assay procedure:- The phenolic content in methanolic extract was determined with the folinciocalteu's reagent (FCR). 1ml of Methanolic extract was mixed with 0.1ml (FCR). After 5mins 2ml of 20% sodium carbonate solution was added the final of the tubes were made up to 10ml with distilled water and allowed to stand for 10mins at room temperature. Absorbance of sample was measured against the blank at 720nm using a spectrophotometer. The results were compared with Gallic acid standard results.

S.NO	Concentration	Absorbance
1	5ppm	0.15
2	15ppm	0.353
3	25ppm	0.586
4	35ppm	0.787
5	45ppm	1.03
6	55ppm	1.283
Slope		0.023
Intercept		0.01266

Table 1: Results of Estimation of Phenolic compounds

Graph:



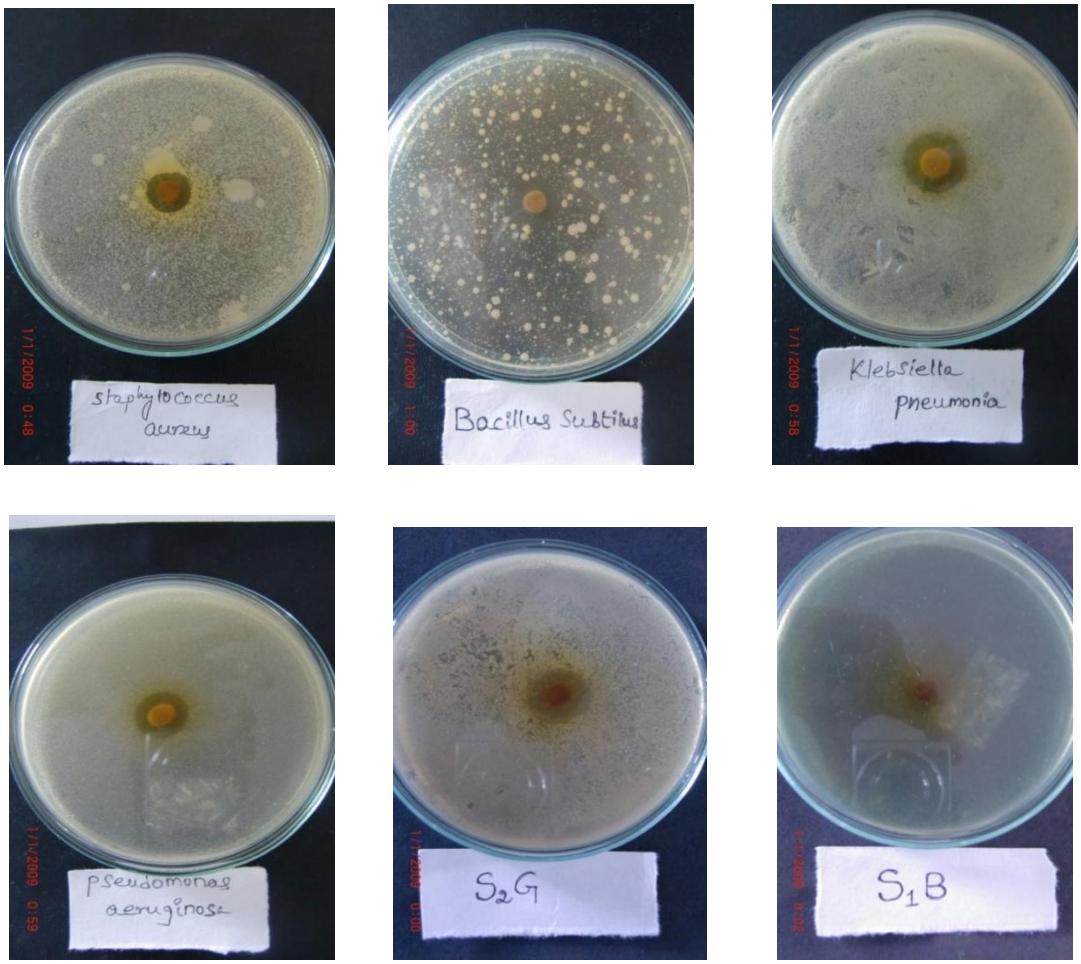
Results and discussions:

The roots of Glycyrrhiza lepidota contain more amounts of Phenolic compounds. On extraction with Methanol more amount of Phenolic compounds were extracted. The amount of Phenolic compounds was found to be 48.66ppm equivalents to Gallic Acid. Results of estimation of Phenolic compounds were given in Table 2. The Phenolic compound in plants having the activity against the growth of numbers of Microorganisms. The anti-microbial activity of Methanolic extract of Glycyrrhiza lepidota in may be due to the presence of high amount of Phenolic compounds. Methanolic extract has shown good effect on both G.+ve and G-ve bacteria. The zones of inhibition of metabolic extract were shown on the table 3.The obtaining results were shown on figures.

S.NO	NAME OF THE STRAIN	TYPE OF STRAIN	ZONE OF INHIBITION IN mm
1	Bacillus subtilus	Gram +ve	12mm
2	Staphylococcus aureus	Gram +ve	14mm
3	Klebsiella pneumonia	Gram –ve	15mm
4	Pseudomonas aeruginosa	Gram –ve	14mm
5	S2G (Bacillus sereus)	Gram +ve	15mm
6	*S1B (Bacillus spericus)*	Gram –ve	7mm

Table 2: Results of antimicrobial test

Figures:



The Methanolic extract of Glycyrrhiza lepidota show more effect on the inhibition of *Klebsiella pneumonia*. It show 15mm of growth inhibition where as the least effect was shown in *Bacillus spericus*.

CONCLUSION

The results of this study reveals that roots of Glycyrrhiza lepidota has antimicrobial activity against the most of the bacteria and this activity may be due to the presence of high amount of Phenolic compounds.

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