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Phyto-chemical screening of Root Extracts of *Glycyrrhiza glabra* by Spectroscopic Methods (UV-VIS Spectrophotometer, FTIR & HPLC)

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ABSTRACT

Glycyrrhiza glabra, commonly known as licorice, has traditionally been used in various medicinal preparations as expectorant, antimicrobial, antiviral, anticancer, anti allergic agent. It has also prominent role in skin whitening and has anti-inflammatory and esterogenic properties. This study aims to the characterization of the bioactive constituent glabridin in plant extract of *Glycyrrhiza glabra* in ethanol, methanol and ethyl acetate using UV-VIS and FTIR and HPLC. A wavelength scan (200-600 nm) by UV-Vis spectrophotometer was performed and the characteristic peaks were recorded. The scan confirmed presence of glabridin with λmax at 281 nm with a small number of additional phytochemicals indicated by the extra peaks. FTIR spectrum of 62% pure glabridin was compared to the spectra shown by the three extracts. Presence of glabridin in all the three extracts was corroborated by HPLC analysis with the retention time of 4.05 minute. Glabridin was also evaluated for its antibacterial activity against *Propionibacterium acne,* causative agent of Acne vulgaris and found to have the detrimental effect at concentration 500, 1000 and 1500 ppm.

Keywords: *Glycyrrhiza glabra*, FTIR, λmax. Phytochemicals, Glabridin, HPLC.

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INTRODUCTION

Medicinal plants provide raw materials for the Indian medical society like *Siddha, Ayurveda and Unani* and also for recent medicines. Indian medicines have thousands of plant-based formulations. Medicinal plants are used for the preparation of millions of preventive and curative medicines. [1-2] Internationally, these plant-formulated drugs are in demand because, they are highly effective, easily available, has negligible toxicity and cheap. These formulations are good substitutes for allopathic medicines. [3-4] These phyto-originated products provide tremendous possibilities for developing new drugs. [5] They could be used for medical preparation in purified or as standardized extracts form of the plant to protect human from several diseases and improve health. Secondary metabolites are the bioactive constituents of the plants and plant synthesize them in root, bark, flowers, leaves, stem and seeds. [6-7] Plants usually produce these chemicals to protect themselves from herbivores and

other predators, and to tide over unfavourable conditions like drought. These phyto-chemicals contribute unique properties to plants. Thus, phyto-chemical analysis of the medicinally important plants is important to explore and evaluate the bioactivity of the compounds. [5, 8]

The 'Grandfather of the herb', Licorice or sweet wood (*Glycyrrhiza glabra* L.; Family: Papilionaceae/ Fabaceae) is native to the southern Europe, North Africa and Western Asia. Traditionally, it is included in various medical preparations as expectorant, antitussive, sweetener, demulcent and laxative. Chinese, Egyptian, Indian, Greek and Roman civilization have been using dried rhizome and root as medicinally important constituents. Now a day, *G. glabra* extracts are frequently used as flavouring agent in preparations to conceal the bitter taste of cold & cough preparations. It also has anti- inflammatory properties that help in detoxification of liver. It is widely used in arthritis and mouth ulcer ailments, to reduce pain. [9-10] In Japan Chronic Hepatitis, Cytomegalovirus (CMV), *Herpes simplex* and Human immunodeficiency virus (HIV) infections are treated with Licorice extracts. Licorice extracts with other drug has benefits in psoriasis and herpetic lesions as it soothe and heal skin eruptions.

G. glabra crude extract contain a variety of phyto-chemicals which could have abundant medicinal properties. Various solvents employed in the extraction of phyto-chemicals are able to dissolve them. Phytochemical analysis of the extract in selected organic solvents can be performed by traditional qualitative and quantitative estimations as well as by Spectroscopic methods (UV-Vis spectrophotometer & FTIR analysis) and chromatography could be utilized to estimate the bioactive constituents separately. [11-15] UV-VIS Spectroscopy shows the peak of the functional group of phyto-chemical represented by λ-max, and same can be compared with standards. [16] Present investigations used ethanol, methanol and ethyl acetate to obtain three separate extracts of *G. glabra* and a comparative study was performed with the help of UV–Vis and Fourier transform infrared spectroscopy and High performance liquid chromatography (HPLC). The results were referenced with the analysis of standard 62% glabridin. *Propionibacterium acne* is the etiological agent associated with Acne vulgaris. It is an aero-tolerant anaerobic Gram positive bacterium, which colonizes and blocks the skin pore. Excessive colonization occurs due to excess sebum production and modified lipid composition in the sebaceous gland at the base of the hair follicle. [17] Even though, Acne vulgaris is not a lethal disorder, it causes significant physiological distress and can be painful. Treatment of acne involves tropical application of retinoids, bezoyl peroxide and antibiotics; erythromycin or clindamycin. Common oral treatments involve uses of antibiotics such as tetracycline and macrolides. Sometimes, depending upon the severity of disease, combinational therapy is preferred which include benzoyl peroxide, retinoids and antibiotics together. [18-19] The treatment with antibiotics of one course typically lasts 3-6 months and resistant to antibiotics is also prevalent in *P. acne.* [20] Thus, there is constant need for the alternative and medicinal plants are the better choice to explore new chemical entity against infectious bacteria. Very few studies have been performed for the anti-acne compound from medicinal plant source. Glabridin from *G. glabra* can be explored for its antibacterial property against *P. acne*.

MATERIALS AND METHODS

Standard glabridin (62% pure) was purchased from Kan Phytochemicals Pvt. Ltd, Sonepat, Haryana, India. It was formulated in 95% ethanol as per the supplier's instructions. Suckers of *Glycyrrhiza glabra* were obtained from the nursery of IGIMS, Patna. It was planted in a pot for further use [Fig. 1a]. Roots of *Glycyrrhiza glabra* [Fig. 1b] were harvested in the month of August from the pot. They were cleaned, shade dried and then dried at 37°C for 4-5 days in an incubator. It was ground in a fine powder by a mechanical mixer with low heating. All reagents used were of analytical grade. For HPLC analysis, HPLC grade chemicals were used. The shade dried roots (dried at about 20°C) were powdered mechanically. 150 ml of solvent was added to the 10 grams of plant powder and kept for 24 hours. The extract was filtered using Whatman No. 1 filter paper and the supernatant was collected. The phyto-constituents gets dissolved in the solvent based on their polarity and are separated by filtration by Whatman No. 1 filter. The obtained residue was re-extracted two-three times with a similar volume of solvent and collected by filtration. The collections were pooled and evaporated (at 30 ± 1°C). All the three extracts were then separately autoclaved and for further analysis. [4, 21-22]

Spectroscopic wavelength scan [UV-Vis Spectrum Analysis and Fourier Transform Infrared Spectrophotometer (FTIR)]

The filtered extract was used in diluted form and wavelength scan was performed in the range of 200 to 600 nm using Shimadzu UV-Vis Spectrophotometer (UV-2550), and the detected individual peaks were documented with their λ-max and plotted with the help of *Origin Prolab* software. For FTIR analysis 100µl of sample loaded on to the probe of FTIR (Brucker). FTIR wavelength acquisition was done from 400 to 4000 cm^{-1} and compared with the reference table available with the instrument software.

HPLC analysis

High performance liquid chromatography was performed on a Shimadzu system (Controller, CBM-20Alite, pump; LC-20 AD, PDA detector, SPD-M20A; Shimadzu, Kyoto, Japan). Samples were diluted and the sample injection volume was kept 20µl for all analyses. For separation, a reverse phase-HPLC system using C18G column with size 250 mm × 4.6 mm, mobile

phase was 70% acetonitrile with 30% water (0.2% acetic acid) were used. The flow rate was kept at 1ml/min throughout the analysis and the acquisition was performed at 25°C for 20 minutes. [23]

Anti *P. acne* activity of glabridin

The antimicrobial activity of glabridin against *P. acne* (ATCC 6919) was determined by well diffusion assay. The assay was performed on Muller Hinton Agar (MHA) in anaerobic condition.

A bacterial lawn was prepared with sterile swab and the wells were punched. Different concentrations (500, 1000, 1500 ppm) of glabridin were used in different well. Levofloxacin (Hi-media) was used as positive control (500 ppm) and solvent used for preparation of glabridin was referred as negative control. Plates were incubated at 37°C. Zone of inhibition was measured in mm and statistically correlated.

RESULTS AND DISCUSSION

The UV-Visible spectra showed major peaks at 206 and 281 nm for 62% pure glabridin. Ethyl acetate extract showed four major peaks respectively at 219, 222, 237 and 281 nm. Ethanol extract showed major peaks at 213, 226, 278 and 315 respectively and methanol extract showed major peaks at 228, 277, 319 nm respectively [Fig. 2, 3]. The λ max peaks of all the three extracts are tabulated in Table 1.

FTIR spectrums of different phyto-chemical groups present in the *G. glabra* extracts were obtained as peak which is the indication of absorption of IR radiation by different functional group. The phyto-chemical groups identified are tabulated in Table 2. FTIR analysis showed phenolic and carbonyl groups, C-H stretch and C-H bends [Fig. 5], which indicates the presence of few flavanoids and other phyto-chemicals in the extracts.

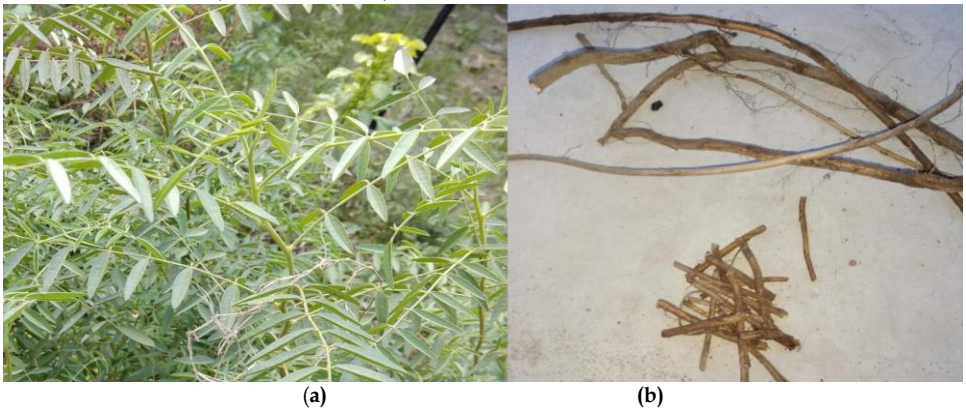
The qualitative HPLC profiles of ethanol, ethyl acetate and methanol extracts were obtained at the wavelength 281 nm. All the profiles were compared with the HPLC profile of the standard glabridin which separated at the retention time of 4.056 minute [Fig 4]. All three extracts showed the presence of glabridin. The HPLC profile of Ethanol extract displayed two important peaks with RT (retention time) of 2.547 minute and 2.723 minute, while that of the methanol extract displayed four important peaks at RT 1.647 minute, 2.052 minute, 2.838 minute and 2.731 minute. The other peaks were obtained at 3.328 minute, 3.851 minute and 4.570 minute. Ethyl acetate extract's HPLC profile displayed a single prominent peak other than glabridin (RT- 4.591 min) at retention time 2.704 min. Two less prominent peaks in ethyl acetate extract HPLC profile were obtained at 1.661 min and 3.878 min.

Table 1: Peak values in UV-VIS spectrum of methanol, ethanol and ethyl acetate extract of *G. glabra*

S. No	Sample	Wavelength (nm)	Absorption peak
1.	Glabridin	206	1.073
		281	0.25
2.	Ethyl acetate extract of *G. glabra*	219	3.048
		222	3.248
		237	3.688
		281	1.984
3.	Methanol extract of *G. glabra*	228	3.192
		277	1.892
		319	1.479
4.	Ethanol extract of *G. glabra*	213	4.498
		226	4.198
		278	2.367
		315	1.608

Table 2: FTIR peak values of glabridin and *G. glabra* extracts

Extract prepared in	Peak Values
Glabridin in ethanol	3392
	1698
	1366
	1232
Ethyl extract	3378
	2927
	1731
	1371
	1235
	977
Ethanol extract	3337
	2973
	1380
	1086
	1044
	879
Methanol extract	3220
	2943
	2832
	1449
	1020



(a) (b)

Fig. 1: *Glycyrrhiza glabra* Plant (a), Roots of *Glycyrrhiza glabra* (b)

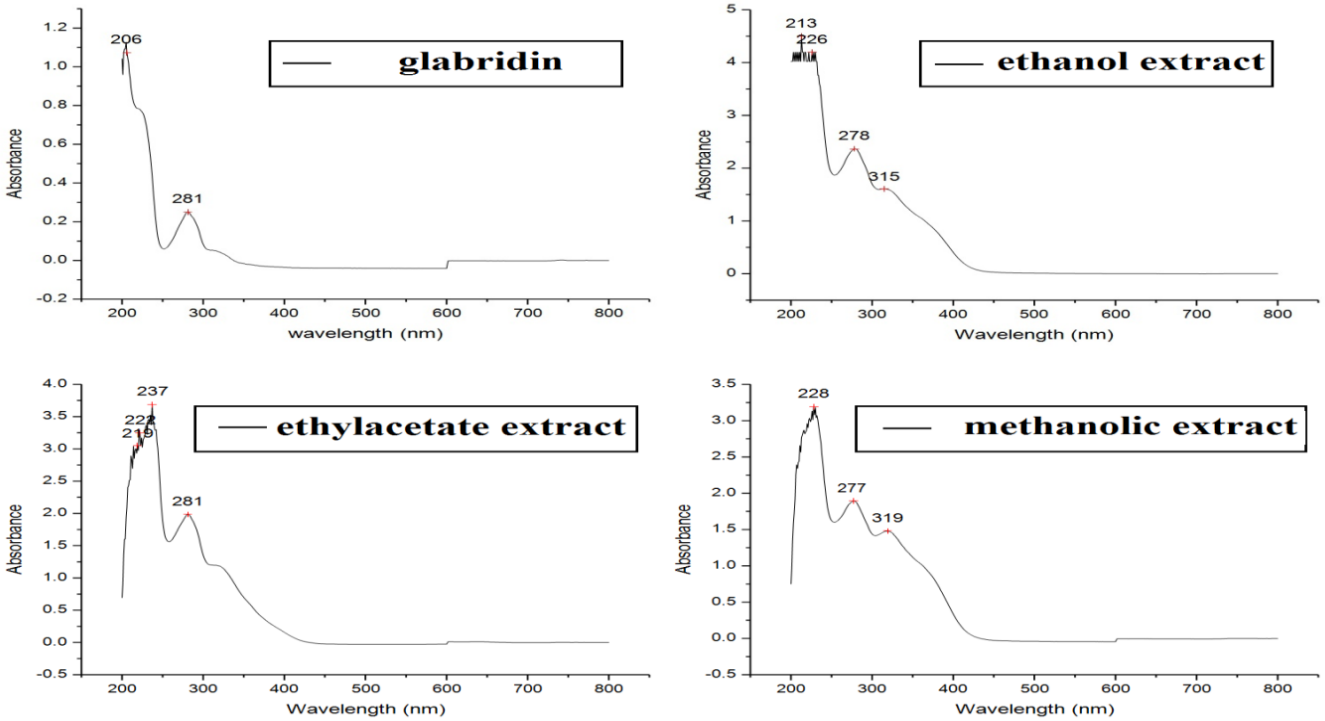


Fig. 2: UV-Vis spectrum of *G. glabra* extracts- Ethanol extract, Ethyl acetate extract, Methanol extract and standard Glabridin

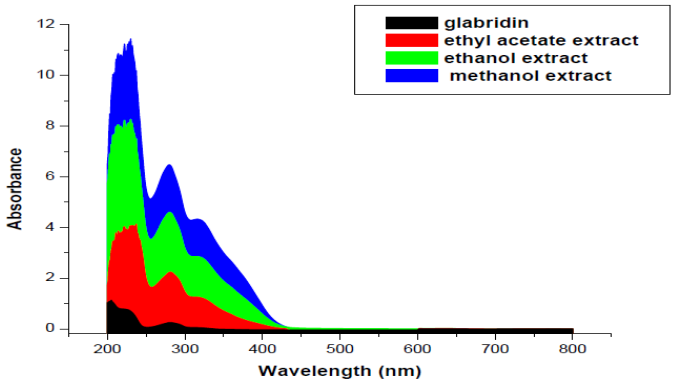


Fig. 3: 3D chromatogram of UV- Vis Scan of Ethyl acetate extract, Methanol extract and Ethanol extracts of *G. glabra* and Stanadard glabridin

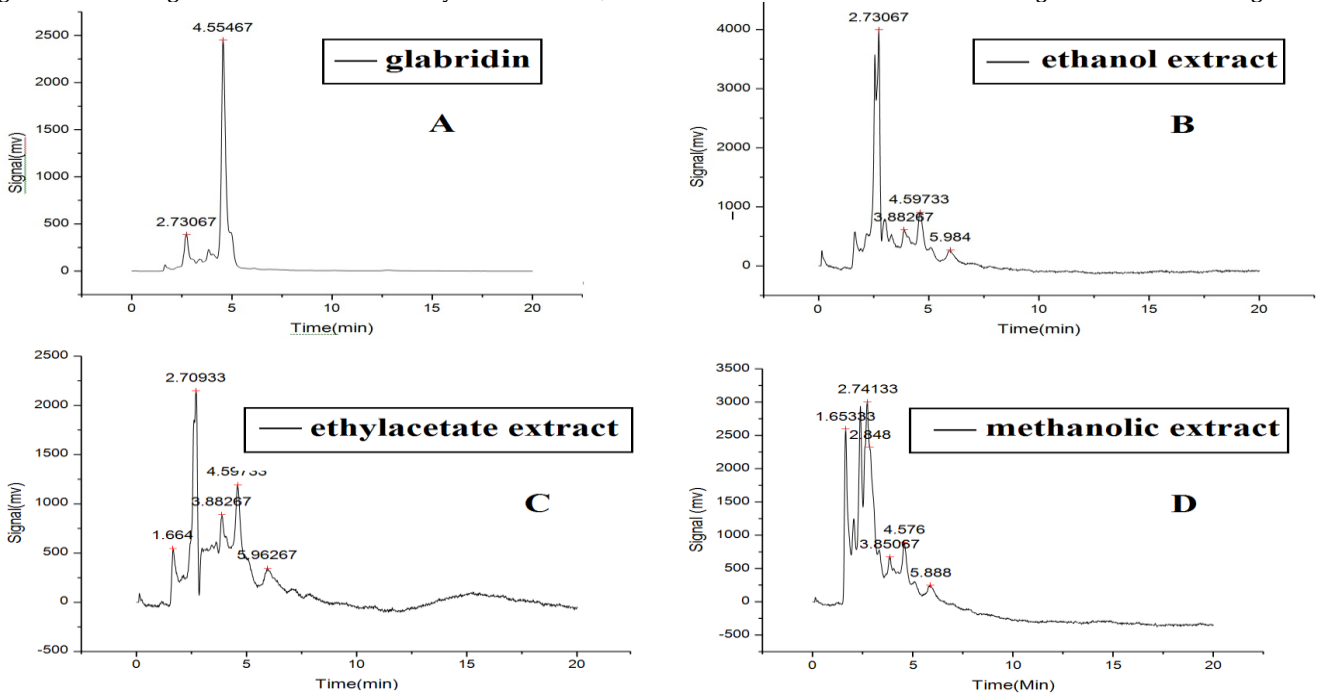


Fig. 4: HPLC chromatogram of the *G glabra* extracts. A- Glabridin, B- Ethanol extract, C- Ethyl acetate extract, D- standard Glabridin

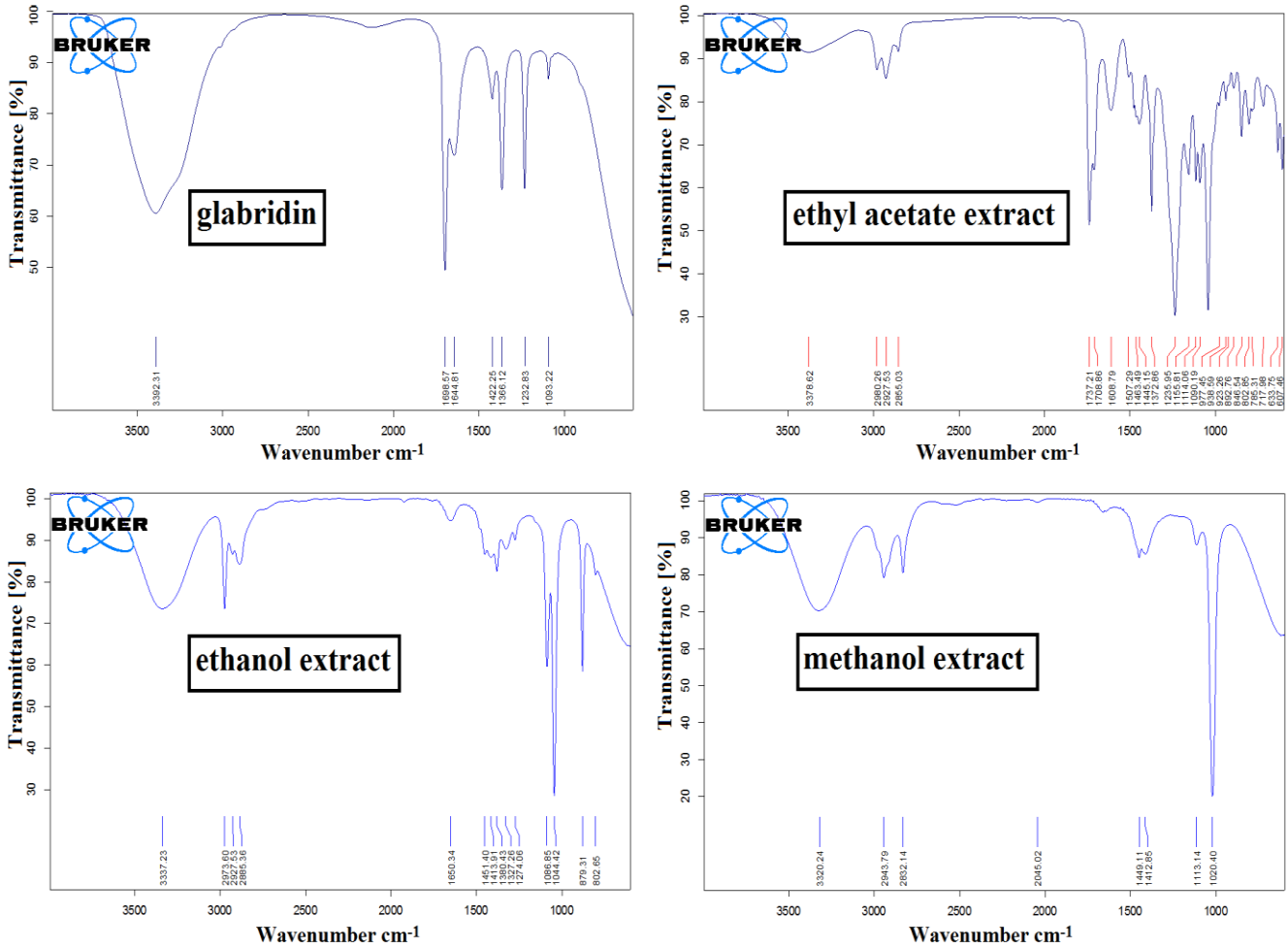


Fig. 5: FTIR spectrum of Root extract of *G. glabra*. A- Glabridin, B- Ethyl acetate extract, C- Ethanol extract, D- Methanol extract

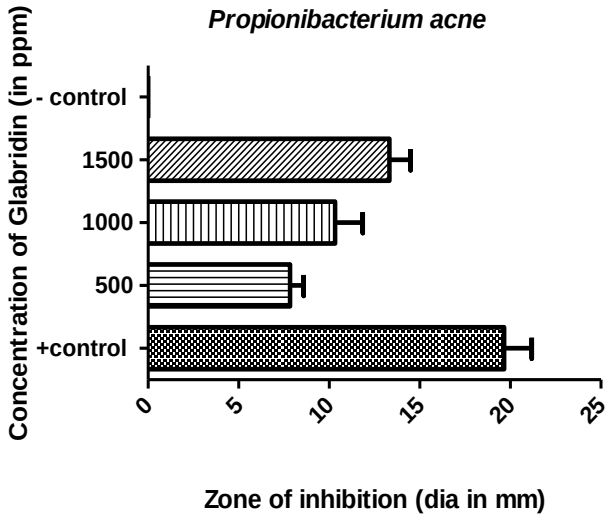


Fig. 6: Zone of inhibition of glabridin against *P. acne*

Ethyl acetate extract showed five peaks in spectrophotometer study while methanol extract had three peaks only. The ethanol extract showed eight peaks. Pure glabridin (62% purity) showed only two peaks at 281 nm and 205 nm. Evidently, the glabridin peak of the standard (281 nm) corresponds with that at 281 of Ethyl acetate extract, 277 in Methanol extract and 278 in Ethyl acetate extract respectively. The observation and results obtained here establish that glabridin may be purified from any of the three extracts by further procedures but more conveniently from Methanol or Ethyl acetate extracts. The latter showed highest concentration of glabridin with greater peak in HPLC at retention time 4.591 min.

The peak values in FTIR varied for different extracts and these values may be referred for –OH group, methylene -CH bend, -CH bend , -C=O group, and -CH stretch functional groups. Our results indicate that the

root extracts of *G. glabra* contain various biologically active functional groups that may belong to diverse compounds which might be responsible for the various biological activities of the root based preparations reported by different workers time to time.

The antimicrobial activity of glabridin from *G. glabra* roots was assessed against *Propionibacterium acne.* The results confirm that glabridin is able to significantly inhibit the bacteria causing *Acne vulgaris* [Fig. 6]. Glabridin could be further analyzed clinically for its application in treatment.

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