ANTIMICROBIAL EVALUATION OF *GLYCOSMIS PENTAPHYLLA* ROOT EXTRACT

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CERTIFICATE

This is to certify that the work entitled, **"ANTIMICROBIAL EVALUATION OF GLYCOSMIS PENTAPHYLLA ROOT EXTRACT"** submitted by **APARANA GUPTA** and **MANU VIKRAM SINGH** in partial fulfillment for the award of degree of Bachelor of Technology in Biotechnology of Jaypee University of Information Technology has been carried out under my supervision. This work has not been submitted partially or wholly to any other University or Institute for the award of this or any other degree or diploma.

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LIST OF ABBREVIATION

1. **D: Distilled water**
2. **M: 70% methanol**
3. **BM: Methanol extract of bark**
4. **CM: Methanol extract of inner part (core)**
5. **C: Aqueous extract of inner part (core)**
6. **B: Aqueous extract of bark**

CHAPTER 1

ABSTRACT

Glycosmis pentaphylla is a perennial shrub widely growing in India, China, Malaya, and was traditionally used in treatment of various skin ailments. Further, modern research has yielded evidences confirming the therapeutic potential of the plant. However, it is not yet understood the exact mode of actions of the plant extract that results in healing up of wounds or skin ulcers. Since skin ulcers are commonly caused by infections, therefore our study focuses on the antimicrobial activity of the crude root-extract of the plant. Therefore, a number of microbes including the skin infecting bacteria and fungus have been considered to check the antimicrobial activity of *Glycosmis pentaphylla* against them. The present investigations confirmed that the aqueous root extract of *Glycosmis pentaphyla* has moderate antimicrobial activity against *Bacillus subtilis*, *E.coli*, *Pseudomonas aeruginosa*, while the methanol (70%) extract of root has the maximum inhibitory activity against the common skin infecting fungus *Trichophyton mentagrophytes*.

CHAPTER 2

OBJECTIVE

Antimicrobial evaluation of *Glycosmis pentaphylla* root extract

CHAPTER 3

INTRODUCTION

Glycosmis pentaphylla (Sanskrit: Ashvashakota; Hindi: Bannimbu) is a perennial shrub which belongs to the family Rutaceae. This shrub grows from 1 to 5meters in height. The leaves usually have 3 to 5 pinnately arranged leaflets. The flowers are small, white, about 6 millimeters in diameter, borne axillary or solitary. The fruit is fleshy, pink or reddish, rounded, 1 centimeter in diameter, and contains a single spherical seed, which is about 5 millimeter in diameter. The fleshy mesocarp is sweet. It is a tetraploid species with the chromosome number (2n) is 72.

Distribution of Plant

Glycosmis pentaphylla is commonly found in thickets and secondary forests at low and medium altitudes, ascending to 1,100 meters altitude, distributed in India, Indo-China and Malaya.

Medicinal Properties of the Plant

Traditionally the plant is known as an effective remedy in curing various kinds of skin diseases and healing of wounds. Also it has been found useful in liver complaints, cough, worms and fever.

(Recorded in the Ayurvedic texts: Charaksamhita)

Modern Research

Chopra et al. (1956) [4] investigated on this plant and reported it to be effective in curing eczema.

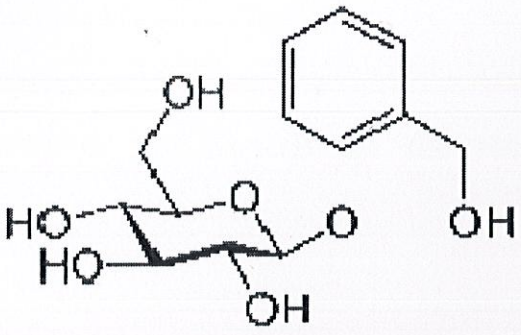
In a recent survey by Shrivastava and Sarkar (2007) [5] based on 20 individuals, afflicted of different types of skin ulcers, it was found that the topical application of aqueous root extract resulted in complete cure of the ulcer in most of the cases.

Bioactive Chemicals

Chopra et al. (1956) [4] based upon a phytochemical analysis found that the plant parts are rich in Glycosmin, which is a glycoside.

The glycosides are the group of organic compounds containing a sugar group bonded through its anomeric carbon to another group via an O-glycosidic bond or an S-glycosidic bond; glycosides involving the latter are also called **thioglycosides**. According to many authors the sugar is bonded to a *non-sugar* for the molecule to qualify as a glycoside. The sugar group is then known as the **glycone** and the non-sugar group as the **aglycone** or **genin** part of the glycoside. Glycosides play numerous important roles in living organisms. Many plants store important chemicals in the form of inactive glycosides; when required, these chemicals are brought in contact with water and an enzyme, and the sugar part is broken off, making the chemical available for use. Many such plant glycosides are used as medications.

(figure 1)



Since *Glycosmis pentaphylla* is found to be effective in healing skin ulcers, it is relevant to mention the essential causes resulting the diseases. Skin ulcers are exposed sores that are often accompanied by the sloughing-off of inflamed tissue. The common causes are the infections, neoplasms, chemical or thermal injury which can be further aggravated by infections. Though the primary mode of action of the plant extract of *Glycosmis pentaphylla* leading to the healing or curing of ulcer is not known, however, what appears to be most probable that it has antimicrobial effect controlling infections in the affected part. The present investigation thus aims to validate the antimicrobial potential of the root extract of *Glycosmis pentaphylla*. The following test microorganisms have been included in the present study

Test bacteria

Bacillus subtilis

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Bacillaceae

Genus: *Bacillus*

Species: *B. subtilis*

Bacillus subtilis, known as the hay bacillus or grass bacillus, is a gram-positive, catalase-positive bacterium commonly found in soil. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endospore, allowing the organism to tolerate extreme environmental conditions.

Bacillus subtilis was used as test bacteria for the standardization of our experimental method.

2. **Bacteria causing secondary infection:-**

1) ***E.coli***

Phylum: Proteobacteria

Class: Gamma Proteobacteria

Order: Enterobacteriales

Family: Enterobacteriaceae

Genus: *Escherichia*

Species: *E. coli*

E.coli is a gram negative bacterium, commonly found in the lower intestine of warm-blooded animals. *E. coli* strains are commonly harmless, but some of them such as serotype O157:H7, can cause serious food poisoning in humans, and are occasionally responsible for costly product recalls.

Intestinal mucosa-associated *E. coli* are observed in increased numbers causing severe bowel inflammation.

3. ***Pseudomonas aeruginosa***

Kingdom: Bacteria
Phylum: Proteobacteria
Class: Gamma Proteobacteria
Order: Pseudomonadales
Family: Pseudomonadaceae
Genus*: Pseudomonas*
Species: *aeruginosa*

Pseudomonas aeruginosa is a gram-negative , rod-shaped bacterium with unipolar motility. It is an opportunistic pathogen for both humans and plants.

Being an opportunistic pathogen for immunocompromised individuals, *P. aeruginosa* typically infects the pulmonary tract, urinary tract, burns, wounds, and also causes other blood infections. It is the most common cause of burn and external ear infections. *P. aeruginosa* may also be a common cause of "hot-tub rash" (dermatitis), caused by polluted water. It commonly causes the burn infections. *Pseudomonas aeruginosa* is also a common cause of post-operative infection in radial keratotomy surgery patients. The organism also causes the skin lesion ecthyma gangrenosum.

Pseudomonas infection of the hand

(figure 2)

Fungus that causes skin ulcers: ***Trichophyton mentagrophytes***

Kingdom: Fungi

Phylum: Ascomycota

Class: Euascomycetes

Order: Onygenales

Family: Arthrodermataceae

Genus: *Trichophyton* (teleomorph: Arthroderma)

Trichophyton is a dermatophyte which inhabits the soil, humans or animals. Related to its natural habitats, the genus includes anthropophilic, zoophilic, and geophilic species.

Trichophyton mentagrophytes cause dermatophytosis and infect the hair, skin, and nails. *Trichophyton* is a keratinophilic filamentous fungus. It commonly invade keratinized tissues and several of its enzymes like acid proteinases, elastase, keratinases, and other proteinases act as the major virulent factors.

CHAPTER 4

MATERIALS AND METHODS

Test Organisms

The bacterial and the fungal strains used in the study were obtained from microbial type culture collection (MTCC), IMTECH, Chandigarh.

BACTERIA

- *Bacillus subtilis*
- *E. coli*
- *Pseudomonas aeruginosa*

FUNGUS

- *Trichophyton mentagrophytes*

Preparation of Root Extract

The air dried root parts of *Glycosmis pentaphylla* were taken for analysis. For surface sterilization the roots were washed 4 to 5 times in a solution of laboline and distilled water. Then roots were transferred to a solution of 0.5% sodium hypochloride and washed repeatedly. Finally roots were washed in distilled water 4 to 5 times.

Both aqueous and methanol (70%) extracts were prepared. For this, the roots were dried and ground in a mixer grinder. 1 gm of the ground root material is mixed with 1 ml of distilled or 1 ml. of 70% methanol [2][3]. The crude root extracts were left overnight and was centrifuged next day. The supernatant was taken for testing the activity.

Agar well diffusion assay

Nutrient agar plates were prepared and subsequently the test organisms were cultured using spread plate technique. The spread plates of the test organisms were incubated for 48 hours to let the strain grow. After the incubation period is over, wells were punctured into the gel plate using a well puncture instrument. 20µl of the supernatant was added to each of the wells. Methanol (70%) and aqueous root extracts were added in separate wells, and the plate is again incubated for more

than 24 hrs. To test the anti bacterial activity. Periodical observations were done to check any development of inhibition zone.

The plates were checked for clear inhibition zones after 24 hrs.

The above standardized procedure was followed for all the test organisms in this experiment.

Ampicillin was used as a positive control. Additionally distilled water and 70% methanol were kept as standard checks.

Broth dilution test

For the Broth dilution test, 100 µl of pure bacterial culture was inoculated in 10 ml nutrient broth solution separately in 4 different vials. The root extracts were added serially to each vial in increasing volume which were 50µl, 100 µl, 150 µl and 200 µl. The broth dilution test was done for both the aqueous and methanol root extract following the same procedure. The broth was left for overnight incubation at 37°c. The change in turbidity was observed and recorded using a spectrophotometer. The optical density of the solution is taken at 595nm [2][6].

Chapter 5

RESULTS AND DISCUSSION

A standard antibiotic agent ampicilin disc was included in each assay as positive control. A clear inhibition zone was observed of 5 mm radius when antibacterial test was done against *Bacillus subtilis* using the ampicillin discs. (figure 5) which indicates the reliability of the experimental procedure.

Antibacterial Assay against *B.Subtilis*

Clear inhibition zones of about 3 mm radius were observed both in case of the aqueous bark and aqueous core root extract when tested against *B.subtilis*. (figure 6). The results were confirmed by the broth dilution tests which showed that aqueous crude root extract has strong antibacterial activity against *B.subtilis* (graph no. 2). Methanol (70%) extracts however has shown relatively less activity indicated by a partially clear inhibition zone as observed in the figure 6.

Antibacterial Assay against *E.coli*

The antibacterial activity of the test sample was observed against *E.coli*, which showed inhibition zone of 2mm radius (figure 7) with both the aqueous extract of the bark and the inner core. The aqueous extract has shown a better activity. The broth dilution test too exhibited antimicrobial activity showing decreasing growth (in terms of decreasing turbidity) with increasing concentration of the extract (graph no. 3)

Antimicrobial Assay against *Pseudomonas aeruginosa*

The aqueous extract of the inner core gave a clear inhibition zone of 2mm radius (figure 8). However methanol (70%) extract showed relatively less activity (both from the core and bark part) which was also found consistent with the results of broth dilution test. (graph no. 4)

Antimicrobial assay against *Trichophyton mentagrophytes*

As seen in figure 9, the methanol (70%) extract of the bark of *Glycosmis pentaphylla* roots showed a clear inhibition zone of radius 2mm, thus establishing the anti fungal activity against the *T.mentagrophytes* strain. In this case the aqueous root extract has shown mild activity however the methanol extract has a strong anti-fungal activity which can be further supported by the results of broth dilution test (graph. 5).

The experiments for each test organism were repeated three times, and the results were found consistent. The antibacterial activities were detected to be influenced by the environmental factors like storage. It showed maximum activity when root samples were analyzed fresh while there was a considerable decline of the activity upon storage (when stored for a period of 6 months).

The major chemical constituents identified in *Gycosmis pentaphyla* are the glycosides. Glycosides are found common in many plant families as well. Triterpenoid glycosides have been isolated from the plant *Pithecellobium racemosum*[1] which showed a moderate antifungal activity against *T.mentogrophytes*, *Candida albicans* and *Saccharomyces cerevisiae*. It can be thus,

reasonably, assumed that various glycosidic compounds in *Glycosmis pentaphylla* may be responsible for the antimicrobial activity of the root extract as observed in the present analysis.

The studies based on human trials [5] showed that the aqueous extract from the root of this plant, when applied topically, worked effectively in healing up of skin ulcers of different types. The present studies yielded supporting evidences that the aqueous extract from the root is more effective in inhibiting the growth of bacteria when compared with methanol (70%) root extract.

CHAPTER 6

CONCLUSION

The present investigation on antimicrobial effect of *Glycosmis pentaphylla* root extract based on agar diffusion assay and the serial broth dilution tests confirmed that the aqueous root extract of *Glycosmis pentaphylla* has a moderate antibacterial property against ulcer causing bacteria like *P.aeruginosa* while methanol (70%) extract is more effective in inhibiting the growth of *Trichophyton mentagrophytes,* the common skin infecting fungus. The scope of the future research lies in identifying the active component(s) attributable to the antimicrobial activity as found with the crude root extract. The information based on further studies in this direction will have valuable implications leading to a complete evaluation of the therapeutic potential of the plant.

Table 1: Antibacterial activities of root extract of *Glycosmis pentaphylla* against different test organisms

	B.subtilis	*E.coli*	*P.aeruginosa*	*T.mentagrophytes*
Distilled water	–	–	–	–
70% Methanol	+	+	+	+
Aqueous extract(bark)	+	+	+	+
Aqueous extract(core)	+	+	+	–
Methanol extract(bark)	+	–	–	+
Methanol extract(core)	–	–	–	+

(+) shows antibacterial or anti fungal activity
(-) shows no antibacterial or anti fungal activity

Nutrient broth of *Bacillus subtilis*
(figure 3)

Streak plate of *Bacillus subtilis*

(figure 4)

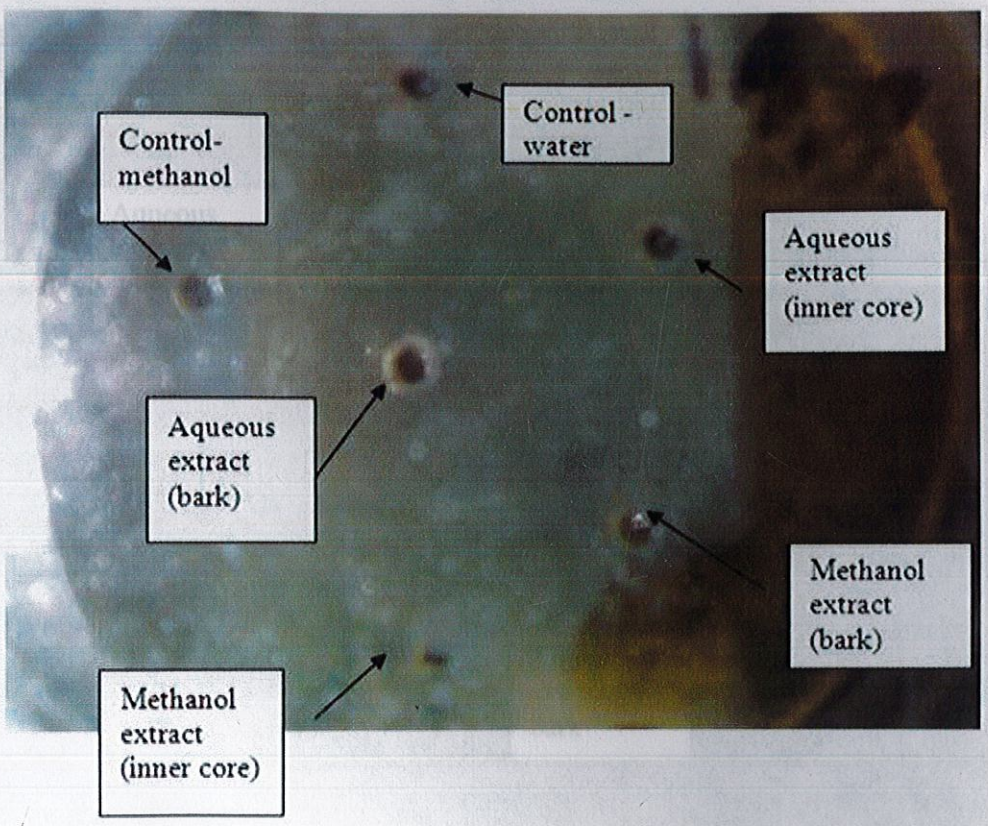
Results for agar well diffusion assay

Result of antibacterial activity of ampicillin discs :a clear inhibition zone is observed for Ampicillin (5mm radius) against the test strain *B.subtilis*.

(figure 5)

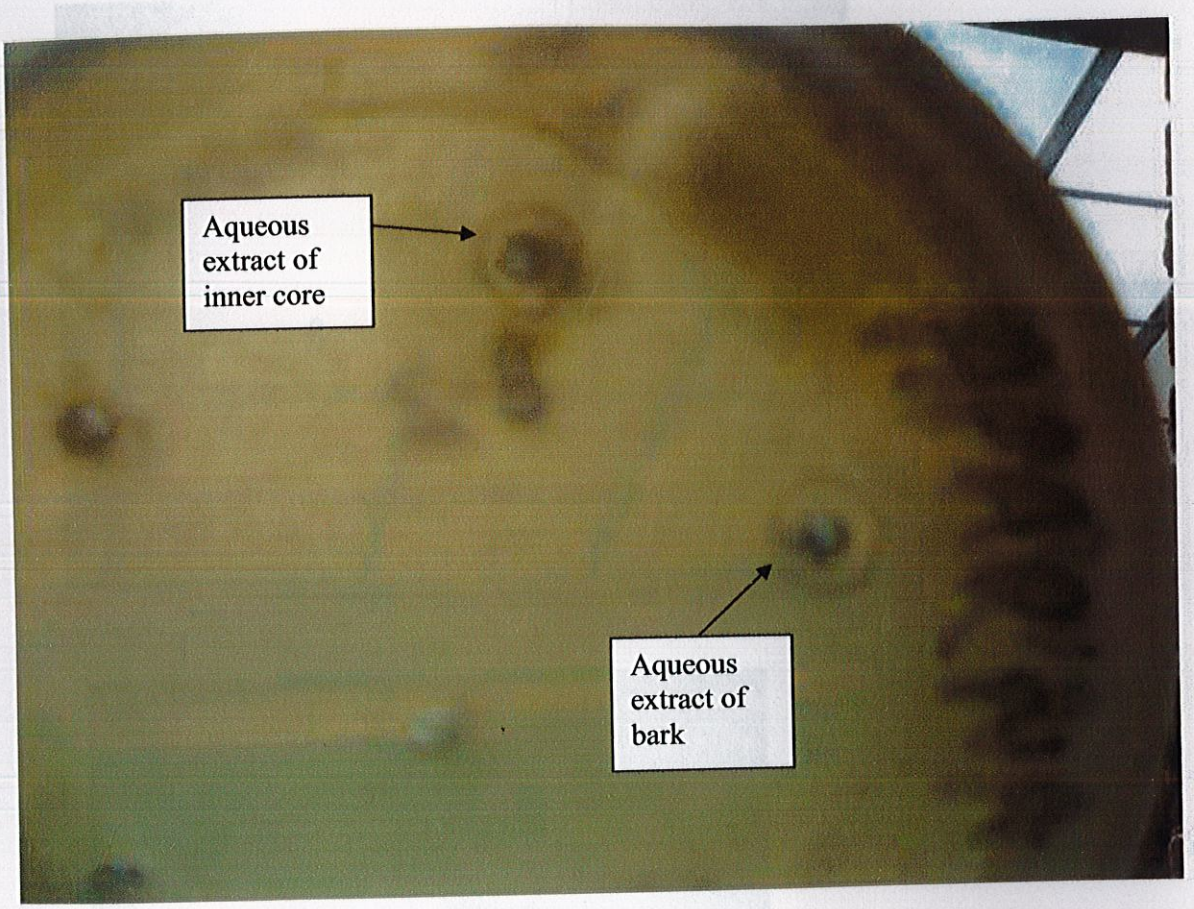
Results of Antibacterial activity of *Glycosmis pentaphylla* against *Bacillus subtilis*

(figure 6)



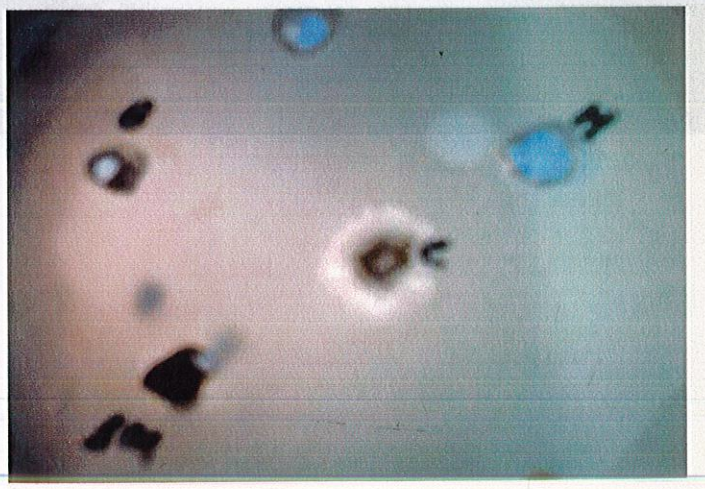
Result of Antibacterial activity of *Glycosmis pentaphylla* against *E.coli*

(figure 7)



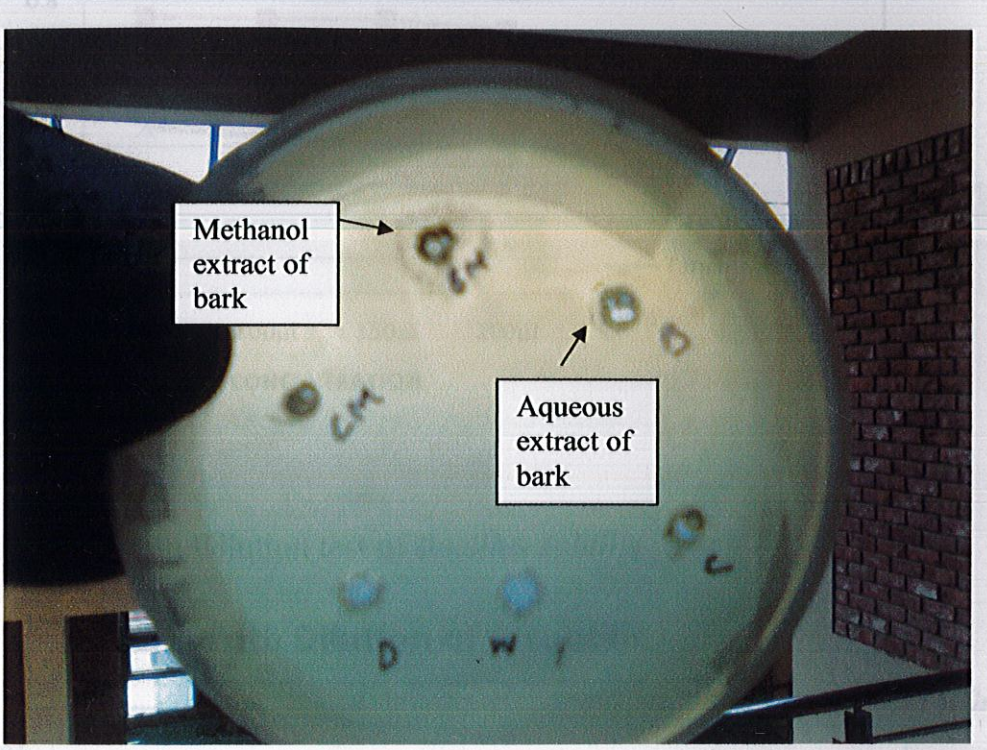
Results of Antibacterial activity of *Glycosmis pentaphylla* against *P.aeruginosa*

(figure 8)



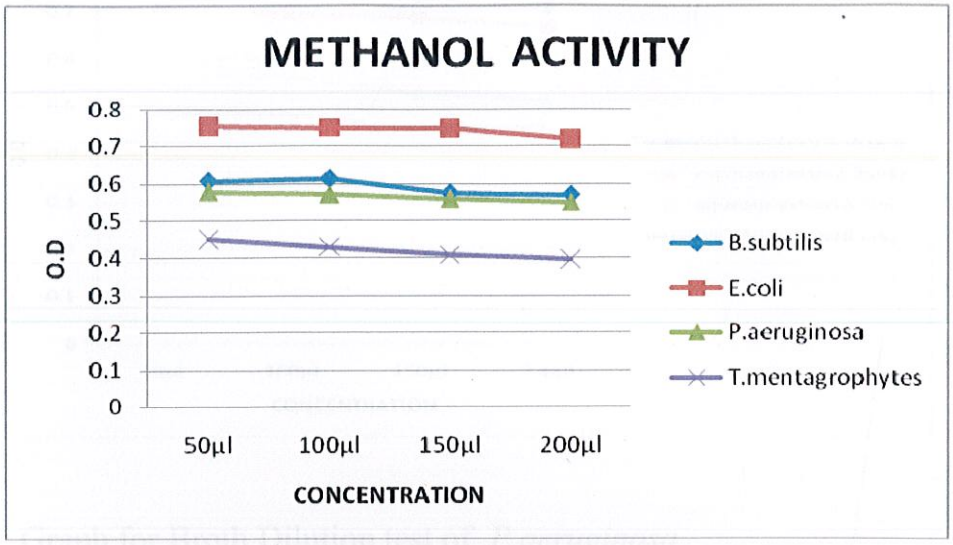
Results of Antibacterial activity of *Glycosmis pentaphylla* against *T.mentagrophytes*

(figure 9)



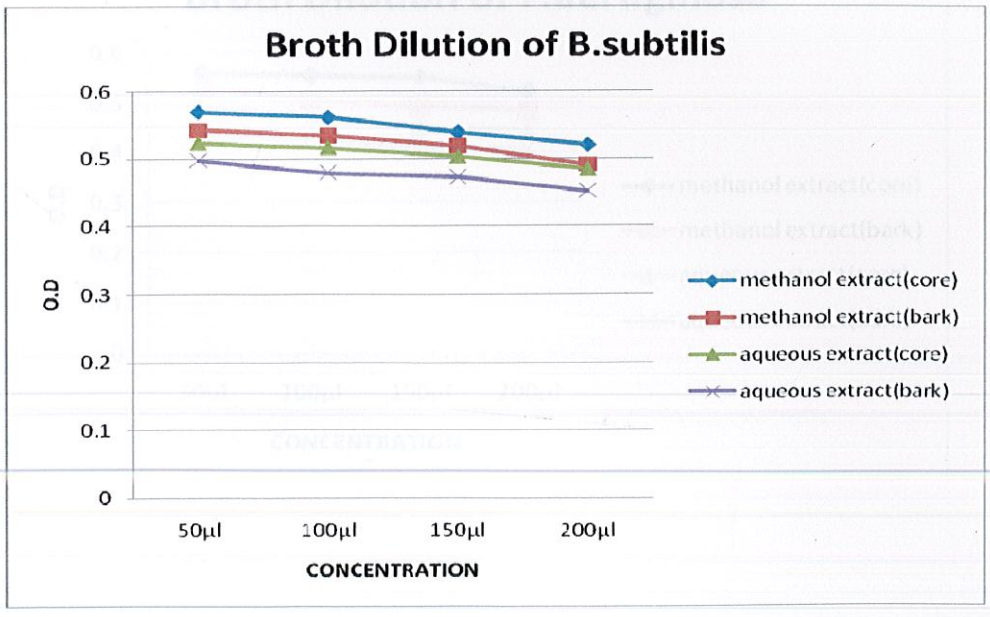
Results of broth dilution test

Graph for antibacterial activity of 70% Methanol



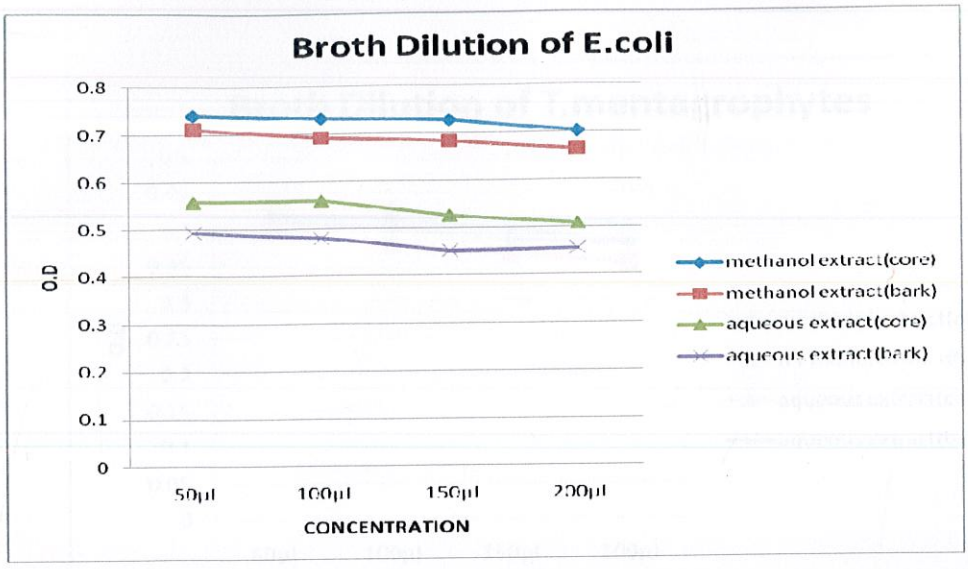
(Graph no. 1)

Graph for Broth Dilution test of *Bacillus subtilis*



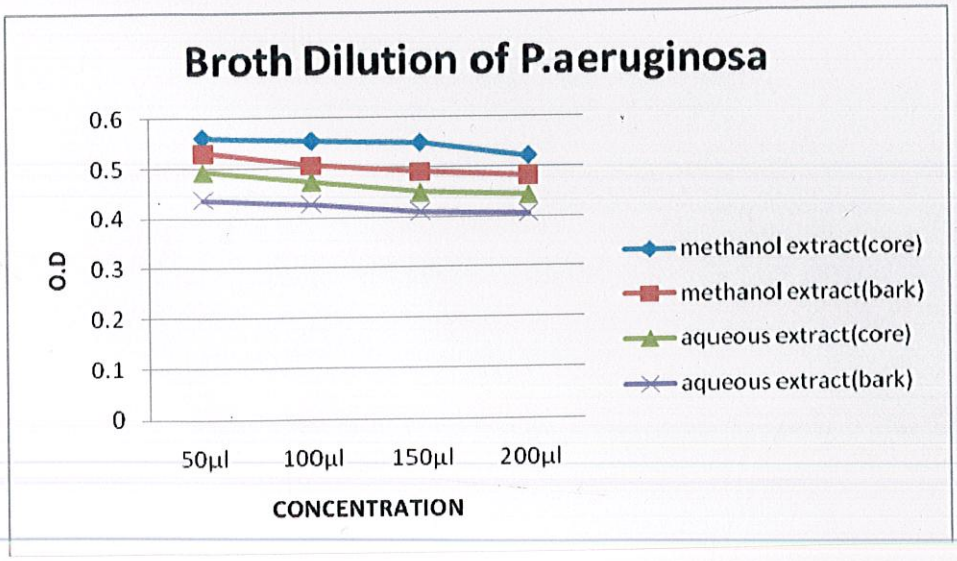
(Graph no. 2)

Graph for Broth Dilution test of *E.coli*



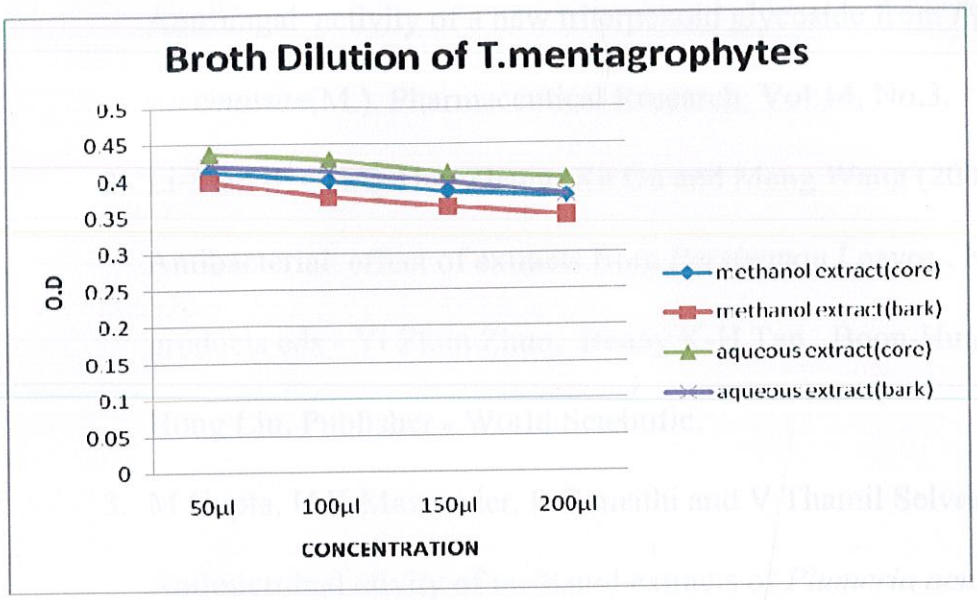
(Graph no. 3)

Graph for Broth Dilution test of *P.aeruginosa*



(Graph no. 4)

Graph for Broth Dilution test of *T.mentagrophytes*



(Graph no. 5)

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