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Toxicity of *Gynandropsis pentaphylla* DC Extracts Against Microbials and Its Phytochemical Profile

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Abstract

Fresh leaves, roots, stems, seeds and seed pods of *Gynandropsis pentaphylla* DC were used in extractions with benzene and ether. Fresh extracts were tested against 6 bacteria and 4 fungi using the well diffusion method. Extracts of roots, stems, leaves, seeds and seed pods were screened phytochemically for the presence of secondary metabolites such as tannin, alkaloid, flavones, sugar, phenolic group, essential oil, amino acids and saponin. All the phytochemicals were observed in all the plant part extracts using both ether and benzene. Among the bacterial cultures, Agrobacterium *tumefaciens* (NCIM 2145) was highly susceptible (28.0±0.3 mm) to both ether and benzene extracts of the leaves of *G. pentaphylla*. Also *A. tumefaciens* showed the highest average zone of inhibition for all extracts. Among the fungi, *Penicillium notatum* (NCIM 747) was highly susceptible (25.0±0.0 mm) to the benzene extracts of *G. pentaphylla* leaves. The extracts of leaves showed highest activity among all the plant parts. Highest activity index was observed for all extracts against the fungus *P. notatum*.

Key Words: Toxicity, Antifungal assay, Bacteria, Fungi, *Gynandropsis pentaphylla*, Phytochemical study.

Introduction

The leaves and seeds of cat's whiskers (*Gynandropsis pentaphylla* DC.) are used in indigenous medicine in many countries (Chweya and Mnzava, 1997; Ajaiyeoba *et al.*, 1998). Cat's whiskers grow as a weed in most tropical countries. In India the common names include *hurhur* and *karaila* (Chweya and Mnzava, 1997). *Gynandropsis pentaphylla* (synonyms: *Gynandropsis gynandra* L. (Briq.) and *Cleome gynandra* L. (Briq) (Chweya and Mnzava, 1997) belong to the plant family Capparidaceae. *Gynandropsis pentaphylla* DC. It is an herb indigenous to the tropical and sub tropical regions. The herb is edible and grows to 60cm high (Dalziel, 1937; Burkhill, 1985; Irvine, 1961; Adjounahun and Ake Assi, 1972).

Hurhur has been used for several years in Indian traditional medical practices. *G. pentaphylla* leaves with a high percentage of vitamin C is taken as a pot herb in soups, fresh or dried (Chweya and Mnzava, 1997). The leaves are used as disinfectants. Inhalation of the leaves also relieves headaches; leaf juice and oil, for earache and eye wash (Chweya and Mnzava, 1997). Seeds have been reputed to have antihelmintic properties and oil is used as fish poison (Walker and Sillans, 1953; Walker, 1953; Chweya and Mnzava, 1997). Sterols are also found in these plants; with lupeol, campesterol and epi-lupeol having been isolated from this palnt (Kondagbo *et al.*,1973; Lakishimi and Chanhan, 1977). In previous studies, the anthelmintic and antimicrobial properties of Capparidaceae plants have been reported (Ajaiyeoba and Okogun, 1996; Ajaiyeoba, 2000) from different countries and in continuation of these objectives on this plant family, the phytochemical, antibacterial and antifungal properties of *Gynandropsis pentaphylla* are presented in the current study for the Indian *G. pentaphylla*.

G. pentaphylla plants have been observed to have insecticidal, antifeedant and repellent characteristics (Akhtar, 1990; Malonza *et al.*, 1992; Pipithsangchan, 1993; Chweya and Mnzava, 1997). The leaves have anti-tick properties. They also have repellent and acaricidal properties for larvae, nymphs and adult *Rhipicephalus appendiculatus* and *Amblyomma variegatum* ticks. Ticks may not be found for a distance of 2-5 m from the plant (Chweya and Mnzava, 1997). The ethanol extract is toxic to insect pests, such as the painted bug (*Bagrada cruciferarum* Kirk) and the diamond back moth (*Plutella xylostella* L.) of cruciferous vegetables. The volatile oils permanently repel the diamond back moth larvae from treated cabbage leaves. The plant has an anti-feedant action against the tobacco caterpillar (*Spodoptera litura* F.). The extract from the mature seeds is toxic to brinjal aphid (*Aphis gossypii* Glov.), and the larvae of *Helicoverpa armigera* (Hubner). The seeds contain phenolic compounds, which are natural products (Jain and Gupta, 1985; Chweya and Mnzava, 1997). Lipids from seeds could be used in soap manufacture (Gupta and Chakravarty, 1957; Chweya and Mnzava, 1997). The biocontrol agent, *Metarhizium anisopliae* (Metsch.) Sorokin (Deuteromycotina: Hyphomycetes) is an extensively studied cosmopolitan filamentous fungus, which is a key regulatory organism of insect populations (Borgio and Sahayaraj, 2007). No reports were available on the activity of *G. pentaphylla* against this entomopathogenic fungus and also against *A. chrococcum*, *L. acidophilus*, *A. tumefaciens*, *P. notatum*.

With these views in mind, the present study was designed to test the toxicity of leaves, roots, stems, seeds and seed pods extracts of indigenous *G. pentaphylla* against the following six bacterial and four fungal species: *Azatobacter chrococcum*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Staphylococcus aureus*, *Aspergillus niger*, *Aspergillus flavus*, *M. anisopliae*, *Penicillium notatum*.

Materials and methods

Preparation of plant extract

Gynandropsis pentaphylla was collected from Rajgurunagar area of Pune, Maharashtra, India in March 2008. About 200g of *G. pentaphylla* leaves were ground in mortar and pestle with 10ml of benzene. Resulting paste collected in a conical flask and allowed to mix properly by placing it in a shaker at 200 rpm for 24 hr. Then the paste was filtered through double layered muslin cloth, the filtrate of leaves in water was used for the further studies. Similar protocol was adapted to prepare leaves extract using ether.

The same procedure was used to prepare the extract from other plant parts like roots, stems, seeds and seed pods. All the extracts were stored at $4^{o}C$ for further analysis.

Phytochemical analysis

Qualitative phytochemical evaluation was carried out to test the presence of alkaloids, flavones, sugar, phenolic group, saponin, amino acid and essential oil in the extracts samples using modified method of Brindha *et al*. (1981).

Tannin: A test solution (500µl) was made with distilled water (500µl), to which 0.01g lead acetate was added. The development of a white turbidity in the precipitate represented the presence of t**annin.**

Alkaloids: A test solution (500µl) was made with 2N HCl (500µl). The aqueous layer was decanted. To the lower layer 2 drops of Mayer's reagent was added. Development of a white turbidity in the precipitate represented the presence of alkaloids

Flavones: The test solution (500µl) was mixed with 100µl of alcohol, 0.02g of paradimethyl amine benzaldehyde and two drops of conc. HCl. Development of red or pink color indicated the presence of flavones

Sugar: The test solution (500µl) was made in a clean test tube, to which 0.01g of anthrone and 3 drops of conc. H_2SO_4 were added. The solution was heated for 1 to 2 minutes. Change of green to purple color was noted to detect the presence of sugar in the sample.

Phenolic group: An alcoholic plant extract (500µl) was prepared in a test tube. Two drops of 1M ferric chloride were added. Appearance of intense color indicated the presence of phenolic groups.

Saponin: A test solution (500µl) with distilled water (2 drops) was prepared in a test tube. The development of a foamy lather indicated the presence of saponin.

Amino acid: A test solution (500µl) made with two drops of 1% ninhydrine in alcohol was prepared in a test tube. Blue or violet color development indicated the presence of amino acid.

Essential oil: A test solution (500µl) made with two drops of 1M alcoholic $K_2Cr_2O_7$ and 3 drops of phenoptheline was prepared in a clean test tube. Soap formation indicated the presence of essential oil.

Assay of Toxicity Levels

Chosen microorganism used

Pure cultures of *Bacillus subtilis* NCIM 2010, *Lactobacillus acidophilus* NCIM 2660, *Agrobacterium*

tumefaciens NCIM 2145, *Escherichia coli* NCIM 2064, *Staphylococcus aureus* NCIM 2120, *Aspergillus niger* NCIM 501, *Aspergillus flavus* NCIM 650, *Metarhizium anisopliae* NCIM 1311, *Penicillium notatum* NCIM 747 were obtained from the National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India. *Azatobacterchrococcum* GCITY 01 was obtained from Genecity Laboratories Pvt. Ltd, Pune.

Media used

Luria agar (LA) (for *B. subtilis, E. coli* and *S. aureus*) potato dextrose agar (PDA) (for *A. niger, A. flavus, M. anisopliae* and *P. notatum*) (HiMedia, Mumbai, India), YEB medium (*A. tumefaciens*), Azotobacter ATCC medium (*A. chrococcum*) and MRS medium (*L. acidophilus*) were used for the bacterial and fungal bio assays.

Antimicrobial agents

Cloroamphenicol (10mg/ml) and fluconozole (10mg/ml) (HiMedia, Mumbai, India) were used as standard antibacterial and antifungal agent respectively. They were included in the current study as standard reference drugs.

Determination of the Toxicity Levels of *G. pentaphylla*

Sterile cotton swabs were dipped in a 24 hour-old *S. aureus* culture. The entire agar surface of LA plate was seeded first in horizontal direction and then in vertical direction to ensure the even distribution of organism over the agar surface using the above swab. The seeded agar surface was allowed to dry for five minutes. Tip of well cutter was sterilized by heating on the blue flame of Bunsen burner and used for the well preparation in the seeded LA plates. Three wells were prepared in each LA plate. As soon as the wells were prepared all plant part extracts (10µl) were poured in each well separately using sterile micro-tips. The same procedure was followed for *A. tumefaciens, B. subtilis, E. coli, A. chrococcum L. acidophilus A. niger, A. flavus, M. anisopliae* and *P. notatum* in their respective plates using 24 and 48 hours of broth culture for bacteria and fungi respectively. Antimicrobial agents [Cloroamphenicol (10mg/ml) and fluconozole (10mg/ml)] were poured as positive control and respective solvents were used as negative control. LA, azotobacter media, agrobacterium media and MRS media plates were incubated at 37±2°C for 24 hrs and PDA plates incubated at 27±2°C for 72 hrs. After incubation the zone of inhibition was measured using HiAntibiotic Zone Scale (HiMedia, Mumbai, India).

Activity Index

The zone of inhibition in extract and the standard antimicrobial agents were used to calculate the activity index.

$$AI = \frac{\text{Zone of inhibition by extract}}{\text{Zone of inhibition by standard antimicrobial agents}}$$

Proportion index

Number of positive results obtained for water, methanol and acetone extract of one plant part was against all the microbials and total number tests carried out were used to evaluate the proportion index.

$$\text{Proportion index (\%)} = \frac{\text{No. of positive results}}{\text{Total number tests}} \times 100$$

Statistical analysis

The zone of inhibition was subjected to cluster analysis to reveal the relativeness of the activity among the human pathogens using STATISTICA/w 5.0. software. A correlation analysis between the pathogens and also different extracts were performed using STATISTICA/w 5.0. software.

Results and Discussion

The phytochemical screening of *Gynandropsis pentaphylla* revealed the secondary metabolites which are of medicinal interest as presented in table 1. *G. pentaphylla* is rich in tannin, sugar, essential oils, amino acid and phenolic group. However, in *G. pentaphylla* seeds extract (both benzene and ether solvents) contained tannins, alkaloids, flavones, sugar, phenolic group, saponin, amino acid and essential oil in plenty of amounts (table 1). Flavons and saponins were comparatively less in this species extracted using both benzene and ether solvents. The variance in the quantitative composition of the secondary metabolite establishes the fact that different plant parts are not likely to have the same medicinal potential. Leaf contained all the secondary metabolites screened. These secondary metabolites are known to exhibit medicinal activity as well as physiological activity (Ross, 2005).

Presence of alkaloids and reducing sugars in leaves and stems were reported by Ajaiyeoba *et al.* (1998). In the present study we also observed the same results in stem and leaves. Apart from both leaves and stems we additionally observed the presence of alkaloids and reducing sugars in seeds, roots and seed pods extracts (ether and benzene) of *G. pentaphylla*. This is not suprising for plants of the Capparidaceae family (Kjaer *et al.*, 1973; Lakishimi and Chanhan, 1977; Ajaiyeoba *et al.*, 1998.).

The results of the antimicrobial activity presented in table 2 shows that both benzene and ether extracts exhibited appreciable antimicrobial properties, inhibiting the growth of all microorganisms. While both benzene and ether extracts of roots not inhibit the growth of *Azatobacterchrococcum*, *Bacillus subtilis*, *Lactobacillus acidophilus*, *Agrobacterium tumefaciens*, *Escherichia coli*, *Staphylococcus aureus*, *M. anisopliae*, *Penicillium notatum*. Two clinical strains of human pathogenic fungi like *A. niger* and *A. flavus* and one entomopathogenic fungi, *Metarhizium anisopliae* and also penicillin producer *P. notatum* were used to find out the antimycotic activity of *G. pentaphylla*. *P. notatum* was the most sensitive; this was closely followed by *A. flavus*, *A. niger* and *M. anisopliae*.

Average zone of inhibition of activities of *G. pentaphylla* extracts against microorganism is presented in Figure 1. The Average zone of inhibition was highest in *Agrobacterium tumefaciens* for both the extracts of all parts of *G. pentaphylla*. Proportion index of antimicrobial activities of *G. pentaphylla* extracts shows the highest activity in leaves extract as presented in Figure 2. The relationship between the sensitivity patterns of different microbials against both ether and benzene extracts used for the

antimicrobial assay had been studied using STATISTICA/w 5.0. software (Table 3). A high correlation coefficient (near 1 or -1) means a good relationship between two variables and its value around zero means no relationship between them at a significant level of $p < 0.05$. More precisely, it can be said that parameters showing $r > 0.7$ are considered to be strongly correlated whereas r between 0.5 and 0.7 shows moderate correlation. In the present study, the resultant matrix reveals strong positive correlation of different bacteria (Table 3) used. ($r > 0.7$).

A data matrix of ten microorganisms and 13 variables (different extracts with two solvents) were used for this cluster analysis, the results are presented in Figure 3. The microbes were separated into two main groups I and II based on their sensitivity: the bacteria, *A. tumefaceiens* with the highest resistant was clustered alone in group I and all the remaining organism with moderate and low resistant were grouped into II (Figure 3). In the group II, only one fungi, *P. notatum* was sub clustered into IIB, which is the second resistant organism (Figure 3). *G. pentaphylla* extracts were more active against fungi than that of bacteria as presented in Figure 4 and Figure 5.

From the result of the antibacterial studies as shown in Table 2, all the extracts exhibited appreciable antibacterial properties, inhibiting the growth of all the bacteria and fungi. The same results were repotted by Ajaiyeoba *et al.* (1998). (Rahman and Choudhary, 1998). In most instances, activities were lesser than the standard therapeutic agent, chloramphenicol as presented in Table 2. The current study was correlated with the previous report (Ajaiyeoba *et al.*, 1998). However, in the current investigation we haven't observed any resistant bacteria to chloramphenicol.

In the antimycotic assay, *P. notatum* was highly susceptible (25.0±0.0 mm) to the benzene extracts of *G. pentaphylla* leaves. Highest activity index was observed generally against *P. notatum*. *M. anisopliae* was moderately sensitive to *G. pentaphylla* extracts. *M. anisopliae* (Borgio and Sahayaraj, 2007) and *G. pentaphylla* (Chweya and Mnzava, 1997; Tvedten, 2007) are active pest control agents, since both are incompatible to each other, it is difficult to use the both pest control agents simultaneously in integrated pest management (IPM). Farmer friendly *A. chrococcm* and *A. tumefecians* are highly sensitive to *G. pentaphylla* extracts. Further studies are needed to evaluate the relationship between these bacteria to *G. pentaphylla* extracts in field condition.

Conclusively, all extracts have displayed antimicrobial activities from present studies. The leaves and seeds of cat's whiskers are used in indigenous medicine in many countries (Chweya and Mnzava, 1997). This has further confirmed the use of this plants in Indian ethnopharmacology for treatment of bronchitis, boils, earache, eye wash, disinfectant and nasal congestion, analgesic, headaches, epileptic fits, facilitate childbirth in pregnant women, stomach-ache, constipation, conjunctivitis, thread-worm infection, chest pains, arthritis, inflammation, neuralgia, rheumatism, localized pains, pus, anaemia, uterine complaints, malaria, pneumonia, head lice and reduce coughing (Burkhill, 1985 Ainsle, 1937; Kerharo and Bouquet,1950; Irvine, 1961; Chweya and Mnzava, 1997; Joy *et al.*, 1998; Ajaiyeoba *et al.*, 1998). There is need for the development of new antibiotics due to acquired resistance, more importantly from natural sources as this delays resistance. *G. pentaphylla* used for the study provide good opportunities for drug development in this area. Further studies are needed to find out the exact compound responsible for the antimicrobial activity using thin layer chromatography, column chromatography and HPLC. Further purification and bioactivity is in progress in our laboratory and will be communicated in future.

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Table 1. Phytochemical screening of *Gynandropsis pentaphylla* extracts.

S. No.	Phytochemical name	Solvent name	Plant parts				
			Leaf	Root	Stem	Seed	Seed pod
1	Tannin	Ether	+++	+++	+++	+++	+++
		Benzene	+++	+++	+++	+++	+++
2	Alkaloid	Ether	+++	++	+++	++	+
		Benzene	+++	±	+++	++	+
3	flavones	Ether	+++	-	+	+++	+
		Benzene	+++	-	++	+++	+
4	sugar	Ether	+++	+++	++	+++	+++
		Benzene	+++	++	+++	++	+
5	Phenolic group	Ether	+++	+++	+++	+++	+++
		Benzene	+++	+++	+++	+++	+++
6	Essential oil	Ether	+++	++	+	+++	+++

		Benzene	++	+++	++	+++	+++
7	Amino acids	Ether	+++	+++	+++	+++	+++
		Benzene	+++	+++	+++	+++	++
8	Saponin	Ether	+++	±	++	+	+
		Benzene	+++	+	+++	++	+

+ + + excellent; + + good; + moderately positive; ± doubtful

Table 2. Antimicrobial activities of *G. pentaphylla* extracts.

Plant parts		Zone of inhibition (mm) (± SEM)									
	Solvent name	*E. coli*	*S. aureus*	*B. subtilis*	*A. tumefaciens*	*L. aceidophilus*	*A. chrococcum*	*A. niger*	*A. flavus*	*M. anisopliae*	*P. notatum*
Leaves	Ether	17±0.1	15±0.0	20±0.0	23±0.1	0.00	0.00	10±0.3	10±0.0	10±0.2	0.00
	Benzene	22±02	23±0.3	21±0.1	28±0.3	0.00	18±0.1	11±0.4	11±0.0	11±0.1	25±0.0
Roots	Ether	0.00	0.00	0.00	13±0.4	0.00	0.00	10±0.3	10±0.4	0.00	0.00
	Benzene	0.00	0.00	0.00	0.00	0.00	0.00	10±0.1	13±0.1	0.00	0.00
Stems	Ether	0.00	0.00	0.00	0.00	0.00	0.00	11±0.0	0.00	0.00	0.00
	Benzene	15±0.1	0.00	12±0.4	0.00	14±0.0	0.00	0.00	10±0.3	12±0.1	13±0.2
Seeds	Ether	11±0.4	12±0.2	13±0.1	28±0.0	14±0.2	0.00	11±0.1	12±0.1	0.00	17±0.1
	Benzene	17±0.2	13±0.0	17±0.0	28±0.2	19±0.3	16±0.2	0.00	0.00	10±0.1	23±0.1
Seed pods	Ether	12±0.0	0.00	11±0.2	14±0.4	0.00	0.00	12±0.2	10±0.3	11±0.3	12±0.3
	Benzene	0.00	0.00	0.00	15±0.0	13±0.1	0.00	0.00	10±0.4	11±0.2	11±0.4
SAA		22±0.2	22±0.0	22±0.1	17±0.1	17±0.0	17±0.0	18±0.0	21±0.0	16±0.0	14±0.0
Control	Ether	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Benzene	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

SAA - Standard antimicrobial agent

Figure 1. Average zone of inhibition of activities of *G. pentaphylla* extracts against microorganism.

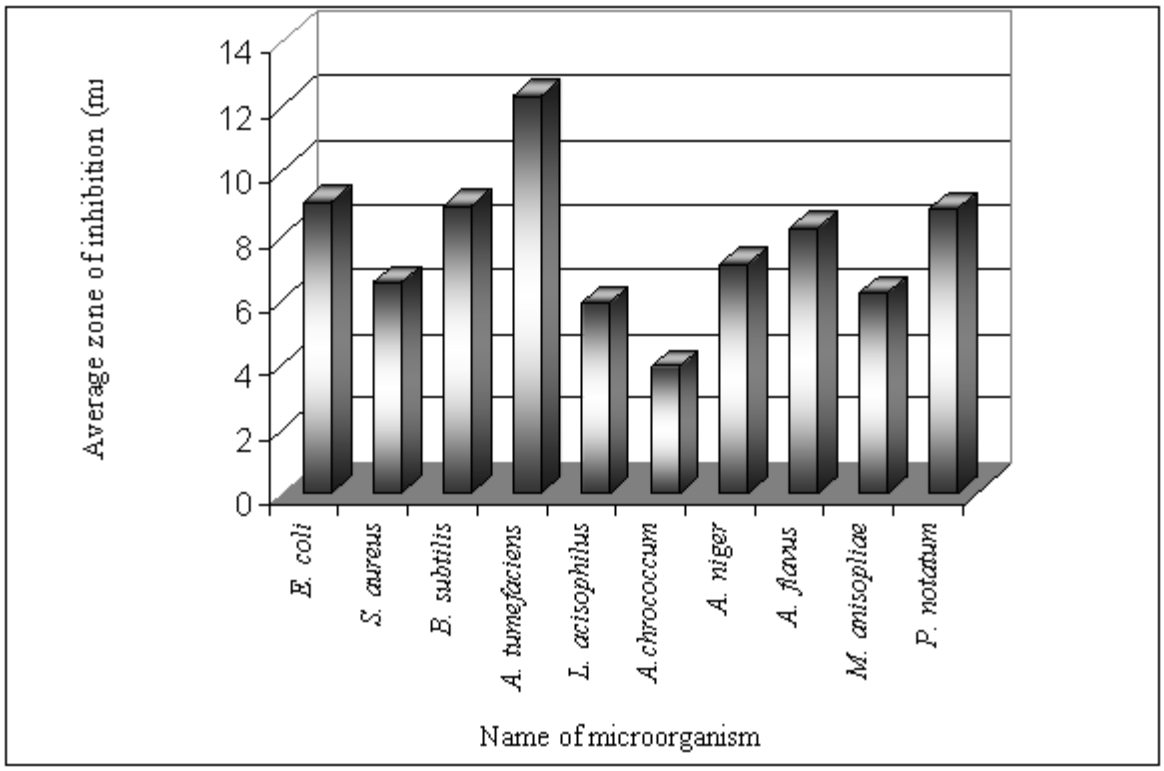


Figure 2. Proportion index of antimicrobial activities of *G. pentaphylla* extracts.

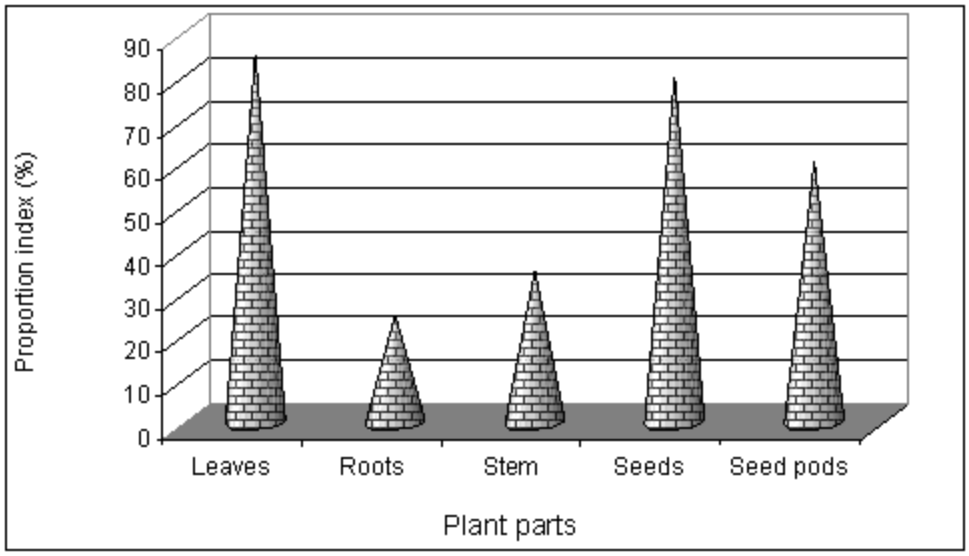


Table 3. Correlation matrix of sensitivity of different microorganism by *G. pentaphylla* extracts.

	E. coli	*S. aureus*	*B. subtilis*	*A. tumefaciens*	*L. aceidophilus*	*A. chrococcum*	*A. niger*	*A. flavus*	*M. anisopliae*	*P. notatum*
E. coli	1.00									
S. aureus	0.66	1.00								
B. subtilis	0.86*	0.90*	1.00							
A. tumefaciens	0.47	0.73*	0.71*	1.00						
L. aceidophilus	0.67	0.43	0.49	0.39	1.00					
A. chrococcum	0.54	0.85*	0.74*	0.55	0.50	1.00				
A. niger	0.37	0.54	0.46	0.32	-0.03	0.34	1.00			
A. flavus	0.45	0.46	0.48	0.33	0.30	0.28	0.67	1.00		
M. anisopliae	0.72*	0.57	0.75	0.44	0.54	0.60	0.20	0.50	1.00	
P. notatum	0.48	0.62	0.68	0.70	0.63	0.71	0.09	0.28	0.62	1.00

*** Marked correlations are significant at p < .05000**

Figure 3. Cluster analysis of sensitivity of 10 different microorganism by *G. pentaphylla* extracts.

a

1

b

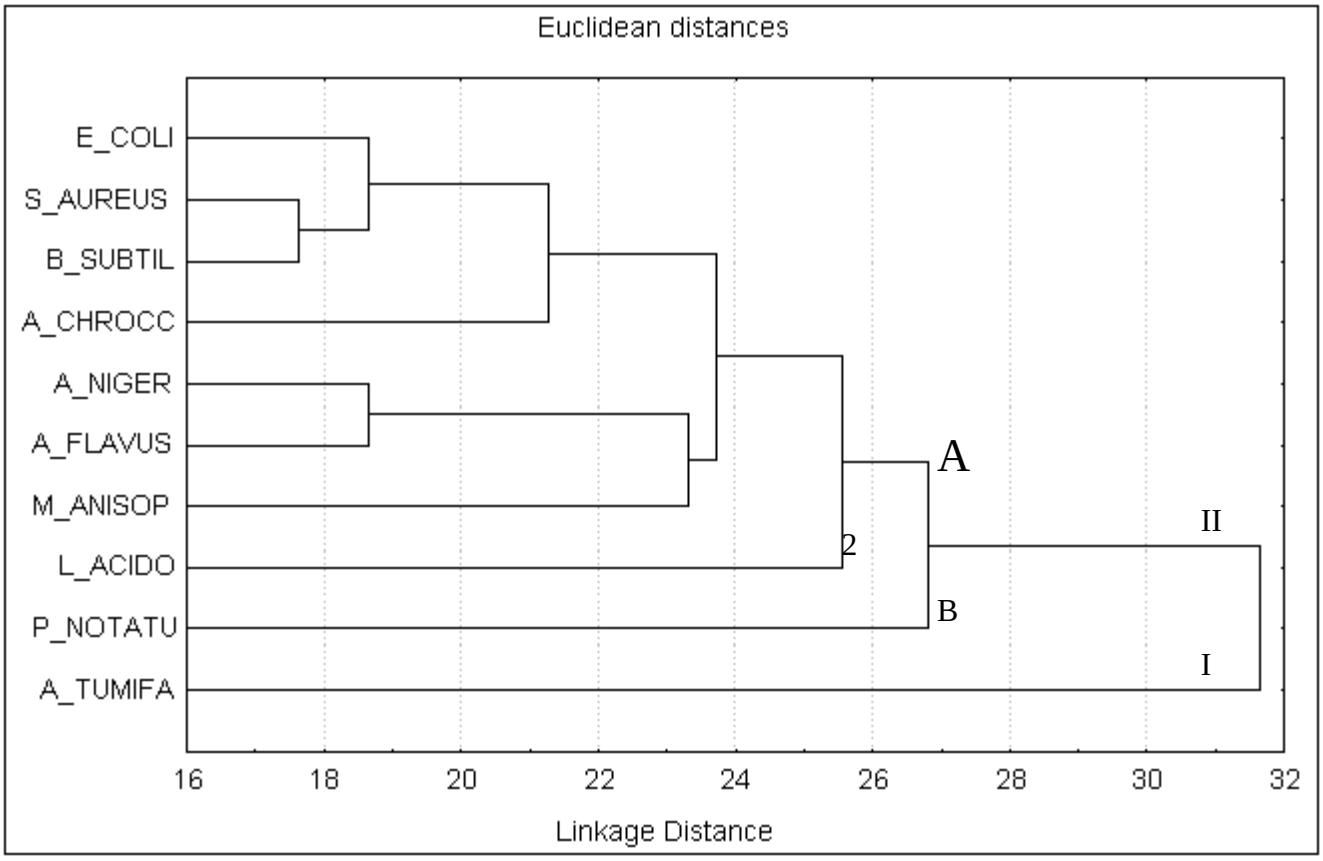
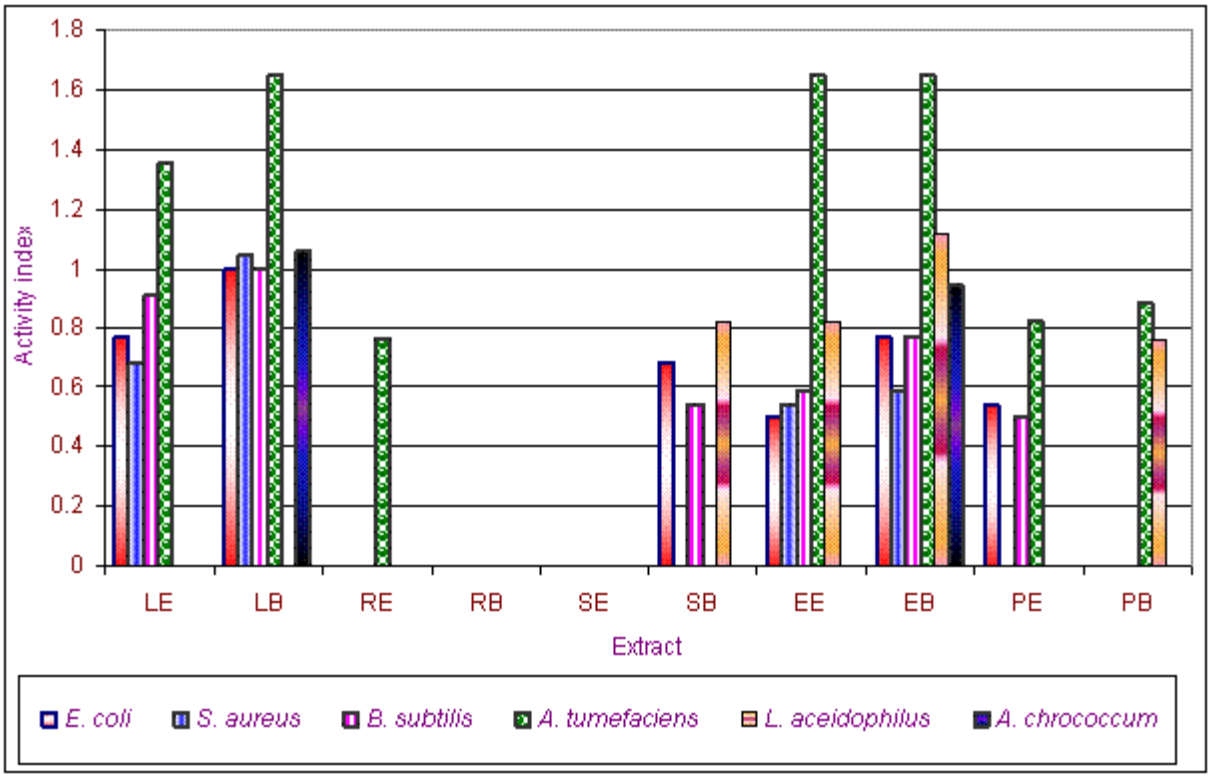


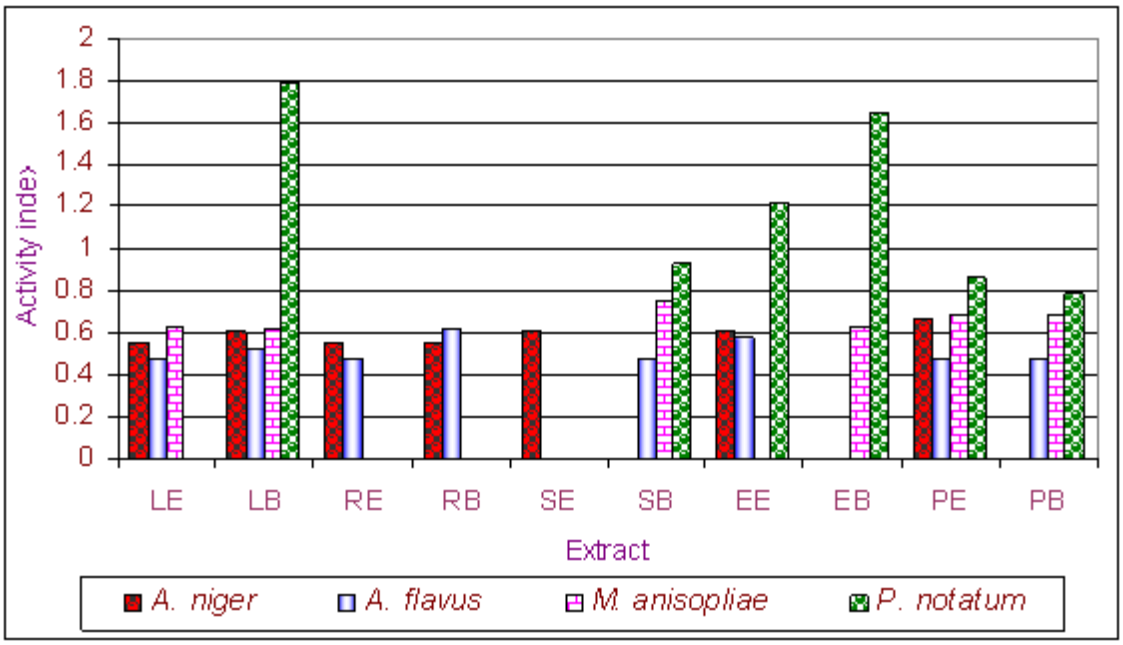
Figure 4. Activity index of *G. pentaphylla* extracts against bacteria.



LE – Ether extract of leaves; LB - Benzene extract of leaves; RE - Ether extract of roots;

RB – Benzene extract of roots; SE - Ether extract of stems; SB – Benzene extract of stems; EE - Ether extract of seeds; EB - Benzene extract of seeds; ; PE - Ether extract of seed pods; PB - Benzene extract of seed pods.

Figure 5. Activity index of *G. pentaphylla* extracts against fungi.



LE – Ether extract of leaves; LB - Benzene extract of leaves; RE - Ether extract of roots; RB – Benzene extract of roots; SE - Ether extract of stems; SB – Benzene extract of stems; EE - Ether extract of seeds; EB - Benzene extract of seeds; ; PE - Ether extract of seed pods; PB - Benzene extract of seed pods.