PHYSIO PHYTOCHEMICAL INVESTIGATION, HPTLC AND ANTI MICROBIAL ACTIVITY OF THE LEAVES OF *GLYCOSMIS PENTAPHYLLA*

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ABSTRACT

To investigate the Anti bacterial and Anti fungal activity of crude extract of Petroleum ether, Chloroform and Methanol obtained from *Glycosmis pentaphylla*. All the extracts were prepared from *Glycosmis pentaphylla* fresh leaves of by hot percolation method in soxhlet apparatus. All the extracts of *Glycosmis pentaphylla* were tested for antibacterial efficacy against *staphylococcus aureus , Streptococci , Klebisella aerogens , Escherichia coli* and antifungal efficacy against *Candida albicans* and *Aspergillus niger*. The Antibacterial and Anti fungal effect produced by Amikacin and Griseofulvin. The Methanolic extract was found to be more effective and showed Anti bacterial and Antifungal activity against the entire organisms tested.

Key words: *Glycosmis pentaphylla*, Antimicrobial activity, Petroleum ether extract, Chloroform extract, Methanol extract.

1.Introduction

Glycosmis pentaphylla (Commom Name : Anam, Ban nimbu, Ashvashakota) an erect shrub of 0.9 to1.8 m. height. Twigs tomentose, terete. Leaves alternate, 3-7 foliolate; the rhachis terete, tomentose, stout up to 18 cm long. Leaflets alternate or sub opposite, 7.5-18 by 3.8-9 cm., elliptic rhomboid or ovate, acuminate or acute, base cuneate usually acute and oblique, entire rarely obscurely toothed, pubescent on both surfaces especially along the nerves, glandular especially on the leaf margin, pellucid-punctate, thinly coriaceous aromatic when crushed, with about 7-12 pairs of lateral nerves (Yoganarasimhan,2000; ThewealthofIndia,1956;Kritikar,1996;Indian pharmacopoeia,1985). Petiole 1.25-5 mm long. Flowers 5mm diameter, yellowish tetramerous, in terminal softly pubescent panicles 10-30 cm long. Juice of the leaves used in liver complaints. Root decoction given for facial inflammations. Wood used in snake-bite. Flowers and leaves used in low fever. Leaves in the form of paste used in skin affections. Leaves used in vermifuge. Decoction of leaves is antidote for eczema, skin troubles. Roots have been used for anti inflammatory. Plant used in anemia, bechic, jaundice. The plant also having antibacterial, antifungal, antiprotozoal activity. Berry 1-1.8 cm long, ovoid, pale orange, verrucose with tufts of short hair or glabrescent when ripe. The leaves of *G.pentaphylla* contain a glycoside glycosmin and alkaloids like glycosin, arborine, glycosminine, arborinine, glycolone. Root bark contains Acridone alkaloids Noracronycine, de-methylacronycine, quinazoline alkaloid ,Glycophymine, Glycosolone, glycolone, Amide, glycomide. Flowers contain alkaloids and amides like glycorine, glycosmicine, benzamide-2-methylamino, also contains carbazole alkaloid Mupamine. The presence of quinazoline bases is significant in connection with the local use of the leaves as a febrifuge(Harbone,1973; Akhtar husain,1992;Chopra,1956).

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2.Materials and Methods

Plant material

Different parts of *Glycosmis pentaphylla* were collected and the parts were cut into small pieces and shade dried. The plant materials were collected in the month of April from Erode district of Tamilnadu. The plant materials were taxonomically identified and the voucher specimens have been preserved in our laboratory for future reference.

Extraction

The whole parts of *Glycosmis pentaphylla* were dried in shade and powdered to get coarse powder. About 225gms of dried powder was extracted with petroleum ether (40°-60°C) by continuous hot percolation using Soxhlet apparatus. The extraction was continued for 72 hours. The petroleum ether extract was

filtered and concentrated to a dry mass by using vacuum distillation. A green waxy residue (2.6gms) was obtained. It was again extracted with chloroform for 72 hours. The chloroform extract was concentrated and a light brown residue (1.9gms) was obtained.

The marc left after the chloroform extraction was dried and extracted with methanol continued up to 72 hours. The extract was filtered and concentrated by vacuum distillation. A greenish brown colour residue (3.9 gms) was obtained.

Physico- Chemical standards

Physico- chemical parameters of the powdered drug such as ash value, extractive value, loss on drying were performed according to the method(The Ayurvedic pharmacopoeia of India,1996; Pharmacopoeia of India,1996;Khandalwal,1999). Extracts were prepared by various solvents by standard methods and percentage of dry extract was calculated in terms of air-dried leaf powder (Table 1, 2, 3).

Fluorescence characteristics

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study(Pratt,1949; Kokoski,1958) (Table 4).

Behaviour of leaf powder with different chemical reagents

Behaviour of leaf of *Glycosmis pentaphylla* with different chemical reagents was performed to detect the occurrence of phytoconstituents along with colour changes under ordinary daylight by standard method(Singh) (Table 5).

Preliminary phytochemical investigation

The qualitative chemical test of various extracts of *Glycosmis pentaphylla* was carried out using standard procedure(Trease,1983;Kokate,1996; Harbone,1973;Plaisted,1958). Carbohydrates, Tannins, Alkaloids, Cardiac glycosides, Flavanoids and Phytosterols were present in all the extracts(Table 6).

Thin Layer Chromatography

About 30gms of silica gel – G was weighed out and it was shaken with 100ml of water to form a homogenous suspension. The suspension was poured into a thin layer chromatography applicator which was adjusted to 0.25mm thickness. 20 to 40 Carrier plates (20.5cm) were laid down for air drying. The plates were kept in the hot air oven at 110°C for one hour to activate the silica gel – G. The plates were stirred in a dry atmosphere and used whenever required. By using the capillary tube the extracts are spotted on the T.L.C plates 2cm above the bottom and in the chromatogram in various solvent systems for different compounds. The spots are developed in solvent system were identified by means of different spraying reagents(Egonstahl,2005;Karel macek,1972) (Table 7).

HPTLC fingerprinting of alcoholic extract

HPTLC instrumentation

Quantitative and qualitative analysis was performed with the help of HPTLC instrument (Sethi,1996;Gandhimathi,2009).The HPTLC system (Camag, Muttenz, Switzerland) consists of

(1) TLC scanner connected with a PC running WinCATS 1.4.3 software under MS Windows

(2) Lincomat V Sample applicator

(3) Photo documentation system Camag

Spotting of samples

The chromatographic estimation was performed by streaking the extracts in the form of narrow bands of 8mm length on the percoated silica gel 60 F254 aluminium TLC plate,at a constant application rate of 15 µl and 100µL was employed with help of Camag 100 µl syringe connected to a Nitrogen tank; using a Camag Linomat V (Camag, Muttenz, Switzerland). The space between three bands was kept 15mm.5µ of 1% concentration solution from each three extracts (Methanol, Choloroform and Petroleum ether) was placed as a spot.

Plate development and chromatographic conditions

After spotting the plate, it is subjected to linear ascending development in a solvent system of Toluene: Acetone in the ratio of 9:1 v/v; at Camag Twin Trough glass chamber, which was saturated with the same solvent system at room temperature just 10minutes prior to development.

Scanning of plate

TLC plate was dried in flowing air at room temperature. Densitometric scanning was carried out using Camag TLC Scanner III between wavelength of 200-450nm with a slit dimension of 6.00 x 0.30mm, with scanning speed of 20nm/s and data resolution was at 100µm/ step. The source lamps for radiation were deuterium and tungsten lamps. The chromatograms were generated using WinCATS evaluation software (Version 1.4.3).

Photo documentation of plate

After the scanning, images of the plate were taken by using different wavelength of lights 254 nm and 366nm with the help of Photo documentation system of Camag. 6 spots with R_f - 0.12,0.14, 0.21,0.52, 0.70,0.84 were observed under 254nm (Fig 1), (Table10),12spotswith R_f-0.12,0.16, 0.21,0.26,0.34, 0.44,0.49,0.58,0.68,0.76,0.84,0.93 were observed under 366nm (Fig2), (Table11), 7 spots with R_f-0.12, 0.14,0.19,0.44,0.59,0.63,0.80 were observed visible light (Fig 3), ((Table 12), 12 spots after spraying of anisaldehyde H_2SO_4(Fig 4), ((Table 13) , 4 spots after spraying $FeCl_3$ (Fig 5), ((Table 14) , 6 spots after spraying Libermann – Burchard (Fig 6), (Table 15) .

Micro organisms Used:

The following bacterial strains used were *staphylococcus aureus (ATCC 25923), Streptococci (ATCC 6633), Klebisella aerogens (ATCC 10240) Escherichia coli (ATCC 25922)*.The fungal species used were *Candida albicans, Aspergillus niger* for the present study.

Evaluation of antibacterial activity

Filter paper disc diffusion method

Agar cultures of the test organisms were prepared as described by Mackeen,1997;Bartner, 1994. Three to five similar colonies were selected and transfered to 5ml broth with a loop and the broth cultures were incubated for 24hrs at 37°c.The extracts were dissolved in Dimethyl sulfoxide with a magnetic stirrer. For screening, sterile 6mm diameter filter paper discs were impregnated with 250 mg of the extracts and then placed in Muller Hinton Agar Medium. The inoculum for each organism was prepared from broth cultures. The concentration of cultures was 1×10^5 colony forming Unit/ml. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the disc indicate the presence of antimicrobial activity. The antibacterial Amikacin (10 mg/m/) and antifungal Griseofulvin (20 mg/ml) were used as reference standards recommended by the National Committee for Clinical Laboratory Standards (Chamundeswari,2004;Khan,2004; Kirankumar Hullati,2004;Sohn,2004; National committee for clinical laboratory standards,2002; Pelczar, 1993).

3.Results and Discussion

Petroleum ether, chloroform and Methanol extracts of *Glycosmis pentaphylla* were tested for antimicrobial activites. Antibacterial and antifungal efficiency of various solvent extract of *Glycosmis pentaphylla* are shown in Table (8,9). The methanolic extract exhibited maximum Antibacterial and antifungal activity when compared with other extracts. In this study, six different bacterial and fungal species were used to screen the possible antimicrobial activites of *Glycosmis pentaphylla.*

As evident from the results antibacterial action of the extracts are more pronounced on Gram (+)ve than on Gram (-)ve bacteria and these findings correlate with the observation of various screening of medicinal plants for anti bacterial activity where the most of the plants showed against Gram(+)ve strain only (Perumalsamy,2000;Lin ,1999).

In addition these results confirmed the evidence in previous studies which reported that methanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants compared to other solvents such as water, ethanol and hexane(Ali,1999;Ahmad,1982;Eloff,1998) .

Table 1: Ash values

S. no	Type of ash	Results
1.	Total ash	10.12 % w/w
2.	Acid insoluble ash	0.63 % w/w
3.	Water soluble ash	1.45 % w/w

Table 2: Extractive value, Percentage yield and colour of extracts

Solvent used	Percentage yield (%)	Colour of extract
Petroleum ether	2.6	Green
Chloroform	1.9	Light brown
Methanol	2.9	Greenish brown

Table 3. Loss on drying

Loss on drying	4.3%

Table 4: Fluorescence characteristics of leaf extract of *Glycosmis pentaphylla*

Powder + Reagent	Colour observed in Ordinary light	Colour observed under Ultra violet light Short (254 nm)	Colour observed under Ultra violet light Long (365 nm)
Powder	Brown	Green	Green
Powder+ 1N NaOH in methanol	Greenish black	Green	Green
Powder+ 1 N NaOH in water	Brownish green	Green	Black
Powder+ 1 N HCl	Brownish yellow	Green	Black
Powder+50% HNO_3	-	Light Green	Black
Powder+50% H_2SO_4	Slight brown	Green	Black
Powder+Methanolic NaOH.dried+ nitrocellulose in aceticacid	Yellowish green	Dark Green	Black
Powder+ 1N NaOH + nitrocellulose in aceticacid	Dark brown	Light Green	Greenish Black
Powder+1N HCl+ nitrocellulose in aceticacid	Black	Light green	Green

Table 5: Behaviour of leaf extract of *Glycosmis pentaphylla*

Reagent	Colour / ppt	Constituent
Powder	Green	
Powder + con. H_2so_4	Brown	Carbohydrate present
Powder + aqueous $Fecl_3$	Bluish black	Tannin present
Powder + Iodine solution	No black	Starch absent
Powder + Aqs. $Hgcl_2$	Blue	Alkaloids present
Powder + picric acid	Yellow	Alkaloids present
Powder + Mg Hcl	Mangoe colour	Flavonoids present
Powder + aqueous $AgNo_3$	Ppt is not formed	Protein absent
Powder + ammonia solution	Pink colour	Cardiac glycoside present
Powder + Aqs. KOH	Pink colour	Cardiac glycoside present
Powder + Aqs. Na nitride	Red colour	Phytosterols present
Powder + Water (shaking)	Foam is not produced	Saponins absent

Table 6: Preliminary Phytochemical test of *Glycosmis pentaphylla*

S.no	Phytochemical tests	Petroleum ether extract	Chloroform extract	Methanol extract
1.	Carbohydrates	+	+	+
2.	Glycosides	+	+	+
3.	Alkaloids	+	+	+
4.	Saponins	-	-	-
5.	Tannins	+	+	+
6.	Proteins and Amino acids	-	-	-
7.	Flavonoids	+	+	+
8.	Phytosterols	+	+	+

+ indicates presence, - indicates absence

HPTLC Finger printing analytical figures

Fig 1 - UV 254nm

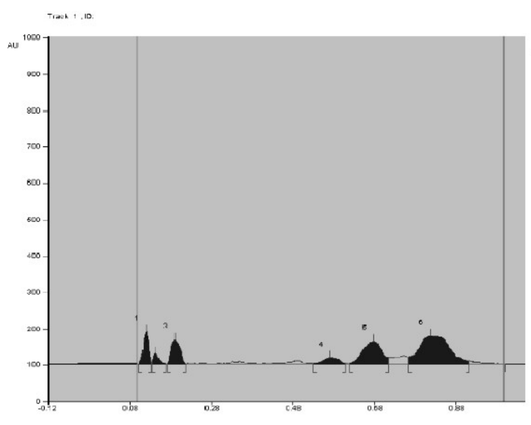

Fig 2-UV 366nm

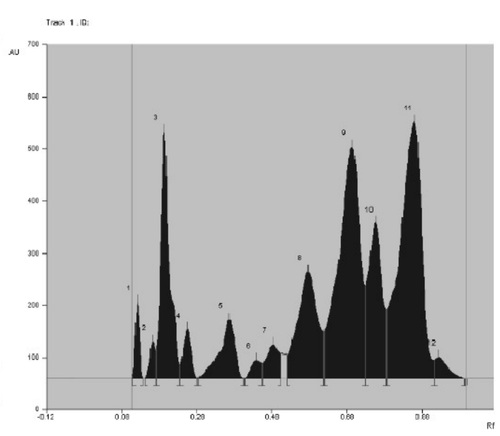

Table 7: Thin layer chromatography of *Glycosmis pentaphylla*

s.no	*Active constituents*	*Mobile phase*	*Spraying reagent*	*Inference*
1	Alkaloids	Methanol : Ammonium Hydroxide 5 : 5	Dragendroff reagent	Orange Brown
2	Cardiac Glycosides	Ethyl Acetate: Hexane 3 : 7	Anisaldehyde Sulphuric Acid	Orange
3	Flavonoids	Butanol : Acetic Acid : Water : Ether 9 : 6 : 1 : 3	Phenol Sulphuric Acid	Greenish Brown
4	Phytosterols	Hexane : Diethyl Ether 32 : 1	Stannic Chloride	Orange Brown Round
5	Carbohydrates	Butanol : Acetic Acid : Water : Ether 9 : 6 : 1: 3	Phenol Sulphuric Acid	Greenish Brown
6	Tannins	Toluene : Acetone 9 : 1	Ferric Chloride	Black

Table 8: Antibacterial activity of different extracts of *Glycosmis pentaphylla*

Microorganisms Bacteria	Zone of inhibition (mm)*			
	Pet. ether extract	Chloroform extract	Methanol extract	Amikacin Standard (10µg/ml)
Gram (-)ve *Klebseila aerogenes*	10±0.9	8±0.5	11.36	14.2±0.5
Escherichia coli.	9.22±0.5	7±0.2	14.4±1.5	16.4±1.5
Gram (+)ve *Stapylococcus aureus*	10.24±0.7	9.02±0.5	13.56±1.3	15.5±1.6
Strepto coocci	11.04±0.7	9.72±0.5	12.44±1.2	14.2±1.7

Zone are Mean ± SD for n = 3

Table 9: Antifungal activity of different extracts of *Glycosmis pentaphylla*

Microorganisms Fungi	Zone of inhibition (mm)*			
	Pet. ether extract	Chloroform extract	Methanol extract	Griseofulvin Standard (20µg/ml)
Aspergillus niger	10±0.9	8±0.5	11.36	14.2±0.5
Candida albicans	9.22±0.5	7±0.2	14.4±1.5	16.4±1.5

Zone are Mean ± SD for n = 3

Fig 3 Visible light

Fig 4 :Anisaldehyde H_2SO_4

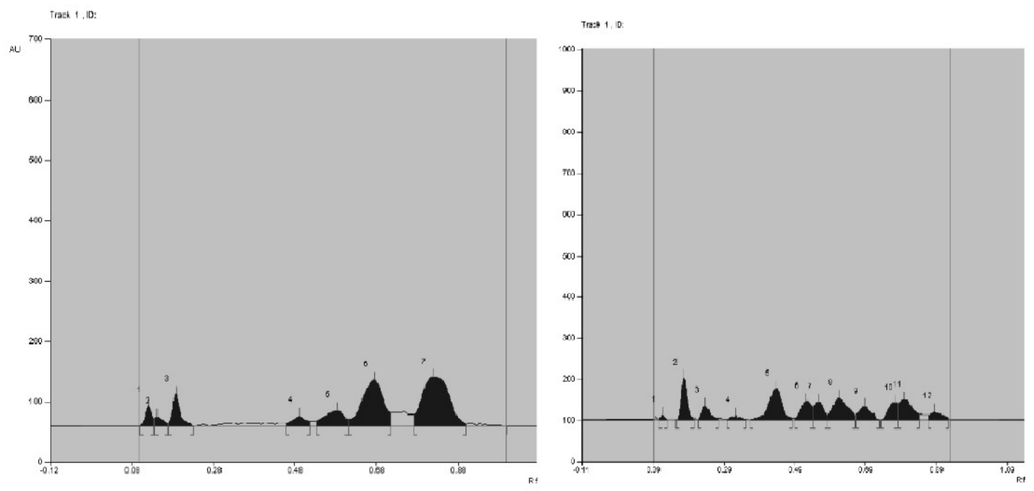

Fig5 $FeCl_3$

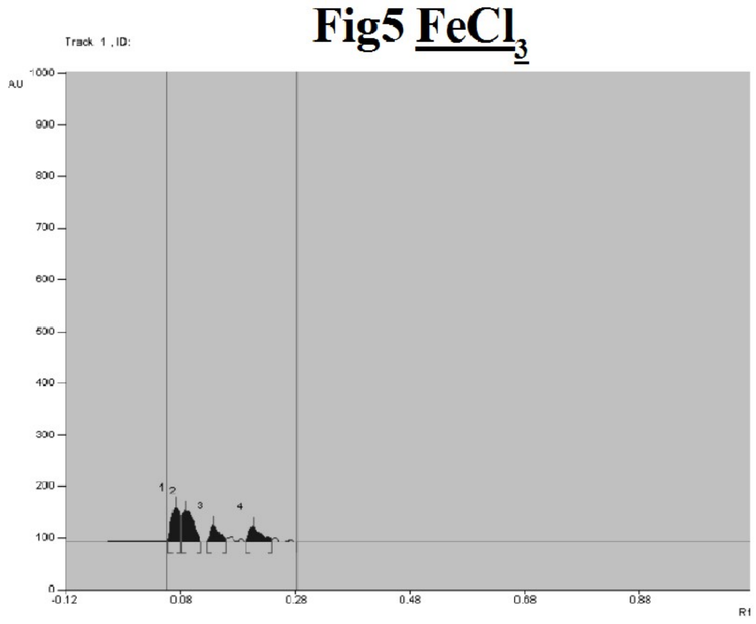

Fig 6 Libermann-Burchard

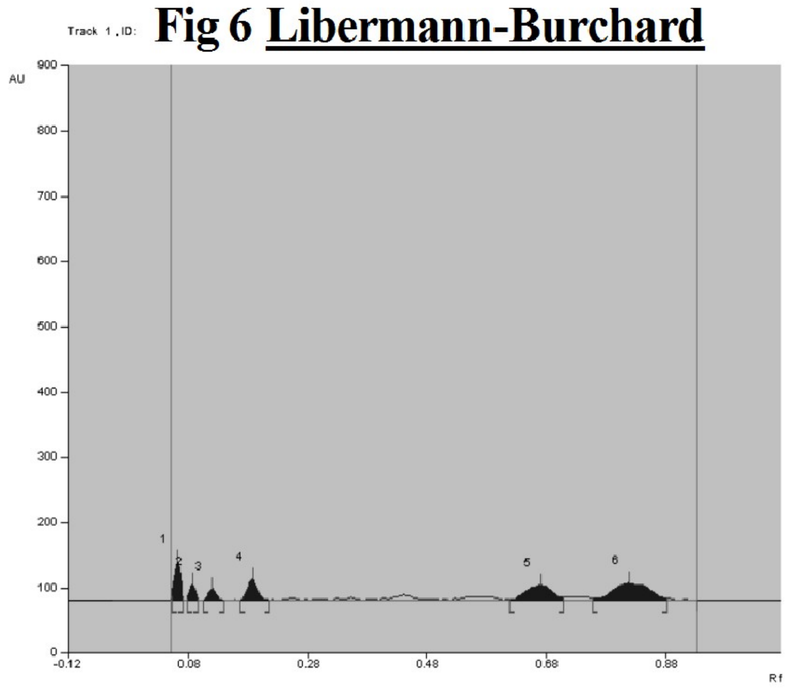

Table 10 - UV 254nm

Peak	Rf value	Area	Area %
1	0.12	943.5	9.39
2	0.14	324.5	3.23
3	0.19	1147.8	11.42
4	0.57	579.6	5.77
5	0.68	2347.4	23.35
6	0.82	4708.2	46.84

Table 11- UV 366nm

Peak	Rf value	Area	Area %
1	0.12	1251.3	1.62
2	0.16	781.8	1.01
3	0.19	8374.8	10.84
4	0.26	1544.0	2.00
5	0.37	3520.6	4.56
6	0.44	711.5	0.92
7	0.49	1685.1	2.18
8	0.58	8381.7	10.85
9	0.70	19608.5	25.39
10	0.76	8405.3	10.88
11	0.86	21877.3	28.33
12	0.93	1088.1	1.41

Table 12 Visible light

Peak	Rf value	Area	Area %
1	0.12	428.0	4.17
2	0.14	257.6	2.51
3	0.19	770.4	7.51
4	0.49	451.0	4.40
5	0.59	1009.8	9.84
6	0.68	3114.3	30.35
7	0.82	4229.7	41.22

Table 13 Anisaldehyde H_2SO_4

Peak	Rf value	Area	Area %
1	0.12	95.5	0.75
2	0.18	1488.2	11.75
3	0.24	665.4	5.25
4	0.33	200.6	1.58
5	0.44	2396.6	18.92
6	0.52	1115.1	8.80
7	0.56	957.2	7.56
8	0.62	1851.5	14.62
9	0.69	907.5	7.16
10	0.78	855.6	6.75
11	0.80	1584.7	12.51
12	0.88	549.4	4.34

Table 14 $FeCl_3$

Peak	Rf value	Area	Area %
1	0.07 Rf	777.0 AU	29.26
2	0.09 Rf	953.7 AU	35.92
3	0.14 Rf	425.3 AU	16.02
4	0.21 Rf	499.3 AU	18.80

Table 15 Libermann-Burchard

Peak	Rf value	Area	Area %
1	0.06 Rf	475.8 AU	14.25
2	0.09 Rf	195.3 AU	5.85
3	0.12 Rf	181.2 AU	5.43
4	0.19 Rf	429.4 AU	12.86
5	0.67 Rf	799.2 AU	23.94
6	0.82 Rf	1257.3 AU	37.66

REFERENCES :

Ahmad I , Mehmood Z and Mohammad F ,Screening of some Indian medicinal plants for their antimicrobial properties,J.Ethano Pharmacol.,1982, 62, 183-193.

Akhtar Husain OP, Viramani, SP, Popali etal, Dictionary of Indian Medicinal Plants, Institute Of Medicinal And Aromatic Plants, 1992, 225.

Ali MS, Azhar I, Amtul Z, Ahmad UV and Usmanghani K, Antimicrobial Screening of fome *Caesalpiniaceae, Fitototerapia;* Vol.70, 1999, 299-304.

Anonymous .The Wealth of India. Raw materials, NISCIR, NewDelhi, India, Vol 5 ,1956, 150-151.

Anonyums, "The Ayurvedic Pharmacopoeia of India ," Part I Vol , 1 st Edition, Govt .of India, Ministry of Health and Family Welfare , (Department of Indian Systems of Medicines and Homeopathy, NewDelhi, 1996, A-53-55.

Anonyums, " Pharmacopoeia of India ,"Vol .2, 4 th ed.,Govt .of India, Ministry of Health, (Controller of publication , NewDelhi, 1996, A-53- 55.

Bartner A, Pfeliffer K. and Batner H , Applicability of disc diffusion methods required by the Pharmacopoeias for testing antibacterial activity of natural compounds , Int J Pharmacogn, 49, 1994, 512-516.

Chamundeswari D, Vasantha J, Gopalakrishna S, Sukumar E, Anti Bacterial, And Anti Fungal Activities of *Trewia Poly Carpa* Roots, *Fitotherpia*, 75, 2004, 85-88.

Chopra RN, Nayar SL, Chopra IC ,Glossary of Indian medicinal plants, National Institute of Science communication , Council of scientific and Industrial research, New Delhi, India, 1956, 126.

Egonstahl, Thin Layer Chromotography, Edn 2, 2005, 883.

Eloff JN , Which extract should be used for the screening and isolation of antimicrobial compounds from plants? J.Ethano Pharmacol .,1998,60,1-8.

Gandhimathi M, Ravi TK, Indian drugs, vol 4, 2009, 150.

Harbone JB, In; Phytochemical Methods, A Guide to Morden Techniques of plant Analysis , Edn 2,Chapman and Hall, New York,1973,85.

Harbone JB. Phytochemical methods.1973.

Indian Pharmacopoeia, vol:2,1985,439.

Karel macek, Pharmaceutical applications of Thin layer and paper chromatography, American Elsevier publishing company, INC,1972,397.

Khan MR., Omoloso AD , Antibacterial Activity of *Galeola Foliate, Fitoterapia,* 75, 2004, 494-496.

Khandalwal KR "Practical Pharmacognosy" (Nirali Prakashan, Edn 6,1999,146-148.

Kiran Kumar Hullati, V.Ravishankar Rai, Anti Microbial Acivity of *Menecylon Malabaricum Leavesm, Fitotherpia,* 75, 2004, 409-411.

Kokate C K, Practical pharmacognosy , Vallabh Prakshan, Pune, Edn.4, 1996, 107.

Kokoski J, Kokoski R and Salma F.J. J.Am.Pharm.Ass 1958,47, 715.

Kritikar KR and Basu BP .Indian Medicinal Plants, International Book Publishers, Dehradun, India, Vol 1,1996.

Lin J ,Opoku A R ,Geheeb-Keller M ,Hutvhings AD,Terblanches SE, Jager AK and Standen J, Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activites, J.Ethano Pharmacol ., 1999,68, 267-274.

Mackeen MM, Ali AM, EI-Sharkawy SH, Salleh MY, Lajis NH and Kawazu K, Antimicrobial and cytotoxic properties of some Malasyian traditional vegetables, Int J Pharmacogn, 1997, 35, 237-243.

National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of Yeast. NCCLS document M27-A2, Wayne Pa.2002, 45-65.

Pelczar M ,Chan ECS and Krieg NR ,In; Microbiology, Int. Edn. Mc Graw-Hill, New York,1993,578.

Perumal Samy Rand Ignacimthu S , Antibacterial activity of some folklore medicinal plants used by tribals in Western ghats of India, J Ethanao Pharmacol., Vol.69, 2000, 63-71.

Plaisted ,Philip .H. Contributions from Boyee Thompron Institute ,1958, 9,231-44.

Pratt RJ , Chase CR .J.Am.Pharm.Ass .1949,38, 324-333.

Sethi PD, HPTLC, Quantitative Analysis Of Pharmaceutical Formulation, Edn 1, CBS Publishers And Distributors, New Delhi, P 3-73, 1996.

Singh VK, Govil Gurdip Singh , " Recent progress in medicinal plants", Vol 1 (SCI Tech publishing LIC Texas), 325.

Sohn HY, Son KH, Kwon CS, Kwon GS, Kang SS, Anti Microbial And Cytotoxic Activity Of 18-Prenylated Flavonoids Isolated From *Morus Alba*.Linn, Phytomedicine, 11, 2004, 666-672.

Trease GE and Evans MC ,Textbook of Pharmacognosy, Balleire, Tindall, London , Edn 12, 1983, 343-383.

Yoganarasimhan SN, Medicinal plants of India, Cyber media, Bangalore, Vol 2, 2000, 264.