IJPSR (2014), Vol. 5, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 09 April, 2014; received in revised form, 10 June, 2014; accepted, 27 July, 2014; published 01 November, 2014

STUDY OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL EVALUATION OF *JATROPHA GOSSYPIFOLIA, SAPIUM SEBIFERUM, KIRGANELIA RETICULATA, PHYLLANTHUS FRATERNUS* AND *PEDILANTHUS TITHYMALOIDES*

U. C. Bapat* and D. R. Mhapsekar

Department of Botany, St. Xavier's College, Mahapalika Marg, Mumbai 400 001, Maharashtra State, India

Keywords:

Jatropha gossypifolia, Sapium sebiferum, Kirganelia reticulata, Phyllanthus fraternus, Pedilanthus tithymaloides, Trichophyton

Correspondence to Author:

Ujwala C. Bapat
Associate Professor and Head,
Department of Botany,
St. Xavier' s College,
1, Mahapalika Marg,
Mumbai - 400 001,
Maharashtra State, India.

E-mail: ucbapat@gmail.com

ABSTRACT: Present investigation deals with the evaluation of the antimicrobial activity of extracts of *Jatropha gossypifolia* Linn., *Sapium sebiferum* (L.) Roxb., *Kirganelia reticulata* (Poir) Baill., *Phyllanthus fraternus* Webster and *Pedilanthus tithymaloides* (Linn.) Poit. against *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus* and *Bacillus cereus and Trichophyton mentagrophytes* and *T. rubrum*. The antibacterial activity was tested by the disc diffusion method and the antifungal (antidermatophytic) activity by the method of Abubacker *et al*. It was observed that only DMSO extracts of the leaves of *K. reticulata* and the flowers of *P. tithymaloides* inhibited the growth of *P. vulgaris* and *S. aureus* with MIC at 200 mg ml^{-1}. The extracts did not inhibit the growth of *T. mentagrophytes* and *T. rubrum.* The phytochemical analysis of these extracts indicated that the tannin and saponin contents were highest in the leaves of *K. reticulata* (6.0 and 12.04 % respectively) whereas, the flavonoid and steroid and triterpenoid contents were highest in the flowers of *P. tithymaloides* (41.20 % and 46.74 %). The MIC values of extracted tannins from the leaves and flowers of *P. fraternus* and *P. tithymaloides* respectively were found to be 40 mg ml^{-1} (against *S. aureus*) whereas, that of extracted tannins and flavonoids from leaves of *K. reticulata* were 50 mg ml^{-1} (against *P. vulgaris* and *S. aureus*). TLC of extracted tannins showed presence of 4 common components, the flavonoids showed 3 and the saponins showed 2 common components. TLC of steroids and triterpenoids did not show presence of any common components.

INTRODUCTION: The use of medicinal plants plays a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(11).4933-41

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(11).4933-41

Jatropha gossypifolia Linn. (= *Jatropha gossypiifolia* L. [2]) is used in the traditional system of medicine for the treatment of various ailments, viz. arthritis, asthma, washing wounds, blood purifier, bronchitis, carbuncles, diarrhoea, dysentery, as an antidote for snake bite, in piles, eczema, fever, gum infections, inflammation, itching, leprosy, stomach ache and ulcer [3]. Bark of root and leaf of *Sapium sebiferum* (L.) Roxb. [=Triadica sebifera (L.)Small [2]] are used against eczema, wounds, edema and snake bites [4]. The leaves of *Kirganelia reticulata* (Poir) Baill. (= *Phyllanthus reticulatus* Poir., family Phyllanthaceae [2]) are diuretic and their juice is used

in diarrhoea in infants. Powdered leaf is applied to sores, burns, suppurations and chafing of skin [5]. *Phyllanthus fraternus* Webster (= *Phyllanthus fraternus* G.L. Webster, family Phyllanthaceae [2]) is useful for treatment of hepatitis, tuberculosis, viral infections, liver diseases, anemia, dysentry and for bacterial infections such as cystitis, prostatitis, veneral diseases and urinary tract infections [6]. The decoction of leaves of *Pedilanthus tithymaloides* (Linn.) Poit. (= *Euphorbia tithymaloides* L. [2]) is used in abdominal disorder. The latex is used in the treatment of venereal diseases and to relieve sprain[7].

The chemistry of the Euphorbiaceae is among the most diverse and interesting of flowering plant families and is comparable to the biological diversity of the family Euphorbiaceae [8]. Various chemical constituents present in *K. reticulata, P. fraternus* and *P. tithymaloides* were earlier reported [6, 9, 10]. Hence, present study was undertaken to correlate the chemical constituents and their antibacterial activity of extracts of the different parts of *K. reticulata*, *P. fraternus*, *P. tithymaloides*, *J. gossypifolia* and *S. sebiferum* against *Escherichia coli* (NCIM No. 2931), *Proteus vulgaris* (NCIM No. 2813), *Staphylococcus aureus* (NCIM No. 5021) and *Bacillus cereus* (NCIM No. 2106) and antifungal activity against the dermatophytes, *Trichophyton mentagrophytes* and *T. rubrum* (causing superficial dermatophytoses).

MATERIALS AND METHODS:

Collection of plant material:

The plant materials were collected from Sanjay Gandhi National Park, Borivili, Vasai, Malad and Matunga, Mumbai and authenticated by studying the morphological characters and by comparing with the Blatter Herbarium specimens. The materials were collected every year from 2009 to 2013 to check the reproducibility of the results. The plants were washed under running water, dried in the shade, powdered and sieved (mesh size 1mm).

Preparation of extracts:

20% extracts of dry and fresh parts of the plants were prepared in different solvents (distilled water, ethanol, methanol, petroleum ether and DMSO) by crushing the material and filtering it through Whatman No. 1 filter paper first and then through Millipore filter (0.45µm). The extracts were prepared in triplicates. For *S. sebiferum*, the extracts of dry material were prepared as the fresh material was not available at the time of study.

Antimicrobial activity Study:

Antibacterial activity assay and determination of MIC of the extracts:

The antibacterial activity was tested by the disc diffusion method [11, 12] with amoxycillin-10 mcg/disc as the antibiotic obtained from Hi-Media and nutrient agar plates were inoculated separately with *E. coli*, *P. vulgaris*, *S. aureus* and *B. cereus*. The MIC of extracts exhibiting inhibition of growth of organisms i.e. diameter of ZOI > 10 was determined by testing 5%, 10%, 15% and 20% extracts using pour plate method[13] (with 6.0 mm wells each with 20µl of extract). The 2 µg of amoxycillin was used for *S. aureus* and 10 µg for *E. coli*, *P. vulgaris* and *B. cereus*. The antibacterial activity of 100% ethanolic extracts of tannins and 20% ethanolic extracts of flavonoids, saponins, steroids and triterpenoids extracted from the leaves of *P. fratenus* and *K. reticulata* and flowers of *P. tithymaloides* was determined by testing 5% extracts using pour plate method. The MIC of extracts of tannins and flavonoids was determined by testing 1%, 2%, 3%, 4% and 5% extracts using pour plate method. All the experiments were replicated thrice for the confirmation of results.

Antifungal (antidermatophytic) activity assay:

The antifungal activity was carried out by the method used by Abubacker *et al* (2008) and Webster *et al* (2008) [14, 15]. One ml of 20 % extracts of fresh and dry plant materials prepared in distilled water (DW) and ethanol (20 % EtOH) were added separately to four ml of SDA medium supplemented with chloramphenicol and cycloheximide [16]. 0.2 ml (about 0.25 –0.4 x 10^6spores/ml) of fungal spore suspension of *T. mentagrophytes* and *T. rubrum* were transferred separately on the plates and spread evenly. 10 mg/ml of Clotrimazole solution (Canesten obtained from Bayer Pharmaceuticals Pvt. Ltd.) was used as standard antifungal agent. The plates were incubated at 30±2 ^{0}C for 21 days and the plates were checked every day for fungal growth (if any). The experiments were repeated thrice for confirmation of results.

Phytochemical screening:

Plant extracts exhibiting activity were screened for the presence of different classes of compounds including tannins, flavonoids, alkaloids, saponin glycosides, steroids and triterpenoids, carbohydrates and proteins[17, 18].

Extraction of tannins, flavonoids, saponins, steroids and triterpenoids:

The tannins were extracted using the method prescribed by Brindha *et al* [19], flavonoids by the method of Edeoga *et al* [20], saponins by the method used by Malu *et al* [21] and steroids and triterpenoids by the method of Bai *et al* [22]. These compounds were extracted and estimated in the leaves of *P. fratenus* and *K. reticulata* and flowers of *P. tithymaloides* showing antibacterial activity.

Separation of compounds extracted by TLC:

The extracted tannins, saponins (ethanolic) and flavonoids, steroids and triterpenoids (20% aq. ethanolic) were applied on TLC plates of silica gel 60 F 254 of E. MerckKGaA with CAMAG Linomat IV HPTLC applicator (syringe). The pre-saturation time was 45 min for tannins, 20 min for saponins and 10 min for flavonoids and steroids and triterpenoids. The run distance was 80 mm. The plates were scanned at 200 nm, 254 nm and 366 nm before derivatization and at 366 nm and 540 nm after derivatization. The R_f values were measured by the CAMAG TLC scanner. The mobile phases and spraying reagents as well as the track positions and the volume of extracts used for TLC is tabulated (**Tables 1** and **2** respectively) as follows:

TABLE 1: MOBILE PHASES AND SPRAYING REAGENTS FOR TLC

Compounds	Mobile phase	Ratio of Mobile phase	Spraying reagent
Tannins[23]	Toluene: Acetone: Formic acid (85%)	6:6:1	Alcoholic $FeCl_3$ reagent
Flavonoids[24]	Ethyl acetate: Formic acid: Glacial acetic acid: Water	10 : 0.5 : 0.5: 1.3	Anisaldehyde sulphuric acid reagent
Saponins[25]	Chloroform: Acetic acid: Methanol: Water	6.4 : 3.2 : 1.2 : 0.8	Anisaldehyde sulphuric acid reagent
Steroids[26]	n-butanol : methanol : water	3 : 1 : 1	10% Sulphuric acid in methanol
Triterpenoids[27]	n-hexane : ethyl acetate	1 : 1	10% Sulphuric acid in methanol

TABLE 2: TRACK POSITIONS AND VOLUME OF EXTRACTS APPLIED

Name of Plant	Tannins		Flavonoids, saponins, steroids and triterpenoids			
	Track no.	Vol. of extracts µl	Track no.	Vol. of extracts µl	Track no.	Vol . of extracts µl
Standards	1	7	-	-	-	-
K. reticulata	2	7	1	2	2	5
P. fratenus	3	7	3	2	4	5
P. tithymaloides	4	7	5	2	6	5

RESULTS AND DISCUSSIONS:

Antimicrobial activity:

Antibacterial activity assay and determination of MIC of the extracts:

The antibacterial activity of the extracts of *J. gossypifolia*, *P. fraternus* and *S. sebiferum* did not inhibit the growth of bacteria except the DMSO extract of the fresh leaves of *P. fraternus* that showed the inhibition of *S. aureus* (the diameter of ZOI = 13mm), the MIC being 150 mg ml^{-1} [28]. Following are the results of the antibacterial activity of the fresh and dried parts of *K. reticulata* and *P. tithymaloides* and their MIC determination.

TABLE 3: ANTIBACTERIAL ACTIVITY OF EXTRACTS OF *K. RETICULATA* AND *P. TITHYMALOIDES* EXPRESSED AS DIAMETER OF ZONE OF INHIBITION (ZOI IN MM)

Organism	Reference antibiotic Amoxycillin +ve Control	Solvent Control	*K. reticulata*						*P. tithymaloides*							
			Entire		Root		Leaf		Entire		Root		Leaf		Flower	
			Fr	Dr	Fr	Dr	Fr	Dr	Fr	Dr	Fr	Dr	Fr	Dr	Fr	Dr
			Aqueous extracts													
E. coli	15.0-21.0	6.0	-	-	-	-	-	-	-	-	-	-	-	-	-	8.0
P. vulgaris	11.0-21.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0
S. aureus	34.0-41.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.0
B. cereus	6.0-10.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
			Ethanolic extracts													
E. coli	18.0-25.0	6.0-7.6	6.0	6.0	6.6	-	6.0	-	-	8.0	-	8.0	-	7.0	8.0	-
P. vulgaris	6.0-18.0	-	7.0	-	-	7.0	10.0	-	-	-	9.0	7.0	-	-	6.0	7.0
S. aureus	37.0-40.0	-	-	-	-	-	8.0	-	-	-	8.0	8.0	-	-	-	8.0
B. cereus	7.0-16.0	6.0	6.0	6.0	6.0	6.0	-	6.0	-	-	-	8.0	-	-	7.0	-
			Methanolic extracts													
E. coli	19.0-23.0	6.0-7.3	6.0	-	6.0	-	6.0	-	-	6.0	-	-	-	-	7.6	-
P. vulgaris	6.0-21.0	-	6.0	-	-	-	8.67	9.0	-	7.0	-	-	-	7.0	-	10.0
S. aureus	35.0-41.0	-	-	-	-	-	6.0	9.0	-	-	-	8.0	-	-	-	10.0
B. cereus	6.0-9.0	-	-	-	-	-	-	-	-	-	-	7.0	-	-	7.0	-
			Petroleum ether extracts													
E. coli	20.0-22.0	6.0	6.0	-	6.0	-	6.0	-	-	-	-	-	-	-	-	-
P. vulgaris	6.0-20.0	-	-	-	6.0	-	6.0	-	-	-	-	-	-	-	-	-
S. aureus	33.0-42.0	-	-	-	-	-	-	-	-	-	8.0	6.0	-	-	-	-
B. cereus	6.0-13.0	-	-	-	-	-	-	-	-	-	-	6.0	-	-	-	-
			DMSO extracts													
E. coli	18.0-22.0	6.0	-	-	-	-	-	-	-	-	-	6.0	-	-	-	-
P. vulgaris	6.0-21.0	-	7.0	10.0	6.0	7.0	11.0	13.0	-	-	-	-	-	-	11.0	12.0
S. aureus	31.0-39.5	-	-	7.0	-	-	-	15.0	-	-	9.0	-	-	-	7.0	16.0
B. cereus	7.0-10.0	6.0	-	-	-	-	-	-	-	-	-	6.0	-	-	`-	7.0

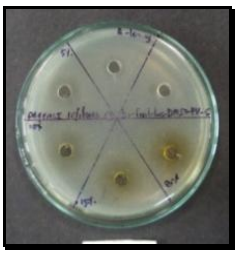

Fig. 1: Fresh leaves of *K. reticulata* (*P. vulgaris*)

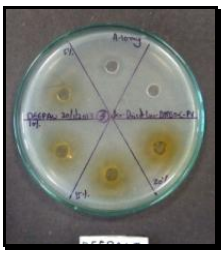

Fig. 2: Dry leaves of *K. reticulata* (*P. vulgaris*)

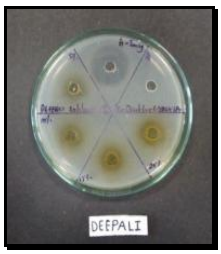

Fig. 3: Dry leaves of *K. reticulata* (S. aureus)

Fig. 4: Fresh flowers of *P. tithymaloides* (*P. vulgaris*)

Fig. 5: Dry flowers of *P.tithymaloides* (*P.vugaris*)

Fig.6: Dry flowers of *P. tithymaloides* (*S. aureus*)

TABLE 4: DETERMINATION OF MIC OF THE EXTRACTS OF LEAVES OF *K. RETICULATA* AND FLOWERS OF *P. TITHYMALOIDES* SHOWING INHIBITORY ACTIVITY AGAINST *P. VULGARIS* AND *S. AUREUS* (ZOI IN MM)]

Amoxycillin	Solvent control	Conc. of DMSO extracts			
		5%	10%	15%	20%
		P. vulgaris			
		Fresh leaves of *K. reticulata*			
11.33	-	8.00	8.33	9.33	10.67
		Dry leaves of *K. reticulata*			
12.00	-	8.00	9.33	10.67	11.67
		Fresh flowers of *P. tithymaloides*			
10.00	-	8.00	8.33	8.67	10.00
		Dry flowers of *P. tithymaloides*			
10.33	-	9.33	10.00	10.33	10.67
		S. aureus			
		Dry leaves of *K. reticulata*			
21.67	-	10.33	10.67	11.33	11.67
		Dry flowers of *P. tithymaloides*			
20.67	-	9.00	9.33	9.33	10.00

The results indicate that the DMSO extracts of fresh leaves of *K. reticulata* inhibited the growth of *P. vulgaris*, whereas that of its dry leaves inhibited the growth of both *P. vulgaris* and *S. aureus* **(Table 3)** and their MIC values obtained **(Table 4)** were 200 mg ml^{-1} **(Fig. 1 to 3)**. The DMSO extracts of fresh flowers of *P. tithymaloides* inhibited the growth of *P. vulgaris* alone, whereas that of dry flowers inhibited the growth of both *P. vulgaris* and *S. aureus* with the diameter of ZOI (16.00 mm) being the highest against *S. aureus* and their MIC values were found to be 200 mg ml^{-1} **(Fig. 4 to 6)**.

Eldeen *et al*(2011) and Shruthi *et al* (2010) obtained inhibitory activity of methanolic extract of dry leaves of *K. reticulata* against *E. coli* and *S. aureus* [29, 30]. The inhibition of growth of *S. aureus* by the ethanolic extract [31] and petroleum ether extract of the dried leaves [32] had also been reported. Mohd *et al* (2006). Adhikary *et al* (2013) reported inhibitory activity of the methanolic extracts of *P. tithymaloides* against *E. coli and S. aureus* [1, 33]. However, no inhibitory activity of the extracts of *P. tithymaloides* was observed against these organisms in the present study. These results are supported by the findings of Chaudhari *et al* (2012) [34].

Antibacterial activity of the tannins, flavonoids, saponins and steroids and triterpenoids extracted from the leaves of *P. fraternus* and *K. reticulata* and flowers of *P. tithymaloides* and the determination of MIC of the same is tabulated in **Tables 5** and **6**.

Fig.7: Tannins of leaves of *P.fraternus* (*S. aureus*)

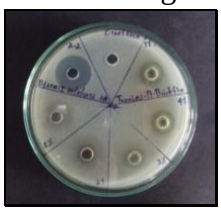

Fig. 8: Tannins of flowers of *P. tithymaloides* (*S. aureus*)

Fig. 9: Tannins of leaves of *K. reticulata* (*P. vulgaris*)

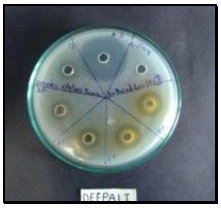

Fig. 10: Tannins of leaves of *K reticulata* (*S. aureus*)

Fig. 11: Flavonoids of leaves of *K. reticulata* (*P. vulgaris*)

Fig.12: Flavonoids of leaves of *K reticulata* (*S. aureus*)

TABLE 5: ANTIBACTERIAL ACTIVITY OF TANNINS, FLAVONOIDS, SAPONINS AND STEROIDS AND TRIERPENOIDS EXTRACTED FROM PLANTS (DIAMETER OF ZOI IN MM)

Organism	Amoxycillin	Solvent Control	Extracted compounds			
			Tannins in 100% ethanol	Saponins in 20% ethanol	Flavonoids in 20% ethanol	Steroids and triterpenoids in 20% ethanol
			Leaves of *K. reticulata*			
P. vulgaris	18.00-23.00	-	10.33	-	11.00	-
S. aureus	26.00-29.00	-	11.67	-	11.00	8.00
			Leaves of *P. fraternus*			
S. aureus	26.00-29.00		10.67	-	-	-
			Flowers of *P. tithymaloides*			
P. vulgaris	18.00-23.00	-	9.00	-	10.00	8.00
S. aureus	26.00-29.00	-	12.33	9.0	10.00	8.00

TABLE 6: DETERMINATION OF MIC OF EXTRACTED TANNINS AND FLAVONOIDS (DIAMETER OF ZOI IN MM)

Organism	Amoxycillin	Solvent Control	Conc. of extracted compounds 1%	2%	3%	4%	5%
			Tannins Leaves of *K. reticulata*				
P. vulgaris	14.67	-	9.00	9.67	10.33	11.33	11.67
S. aureus	24.33	-	9.33	10.67	11.67	13.33	13.67
			Leaves of *P. fraternus*				
S. aureus	26.67	-	9.00	9.33	10.00	11.00	11.00
			Flowers of *P. tithymaloides*				
S. aureus	24.00	-	9.33	10.33	10.67	12.00	12.00
			Flavonoids- Leaves of *K. reticulata*				
S. aureus	24.00	-	8.67	9.00	9.67	10.67	11.00

It was observed that among the extracted compounds tested, the ethanolic extracts of tannins from the leaves of *P. fraternus* and flowers of *P. tithymaloides* inhibited the growth of *S. aureus* with the highest diameter of ZOI of 12.33mm for the latter and their MIC values were found to be 40 mg ml^{-1} **(Fig 7** and **8)**. The ethanolic extracts of tannins and flavonoids from the leaves of *K. reticulata* showed inhibition of growth of *P. vulgaris* and *S. aureus* **(Table 5)** and their MIC values were observed to be 50 mg ml^{-1} **(Table 6) (Fig 9** to **12**).

Antifungal (antidermatophyte) activity assay:

It was observed that none of the extracts tested inhibited the growth of *T. mentagrophytes* and *T. rubrum*. Adejumo *et al* (2009) had reported the inhibitory activity of fresh leaves of *J. gossypifolia* against *T. mentagrophytes* and *T. rubrum*[35].

Phytochemical screening:

The phytochemical tests of the DMSO extracts of fresh and dry leaves of *K. reticulata* and that of flowers of *P. tithymaloides* showed the presence of tannins, flavonoids, saponin glycosides, steroids and triterpenoids, carbohydrates and proteins in the present study. The same compouds were reported to be present in the DMSO extracts of fresh leaves of *P. fraternus* in the previous studies [28]. Presence of alkaloids, tannins, flavonoids, glycosides, saponins, steroids and triterpenoids in the dry leaves of *K.* reticulata had been reported [Narasimhudu *et al* and Gopinath *et al* (2012)] [36, 37]. Kantamreddi *et al* (2010) reported presence of steroid and triterpenoid in the methanolic extract of dry leaves of *K. reticulata* and absence of alkaloid and flavonoid of the same[38]. The DMSO extract of leaves of *K. reticulata* in the present study exhibited presence of all the tested chemical constituents except alkaloids.

Raveen *et al* (2012) had shown presence of flavonoids, alkaloids, steroids, tannins, saponins, proteins, carbohydrates and glycosides in the ethanolic extracts of *P. tithymaloides* [39]. Prakash *et al* (2013) observed the presence of tannins and absence of flavonoids, saponins, terpenoids and steroids in the aqueous extract of dry leaves of *P. tithymaloides* [40]. Present study showed the presence of all the tested phytoconstituents except alkaloids in the DMSO extracts of flowers of *P. tithymaloides*. There are no reports on the phytochemical analysis of flowers of *P. tithymaloides*.

Estimation of tannins, flavonoids, saponins, steroids and triterpenoids:

Since the crude extracts of leaves of *K. reticulata* and *P. fraternus* and flowers of *P. tithymaloides* showed presence of tannins, flavonoids, saponins, steroids and triterpenoids, these compounds were extracted and their total content was determined **(Table 7)**.

TABLE 7: TOTAL TANNINS, FLAVONOIDS, SAPONINS AND STEROIDS AND TRITERPENOIDS IN *K. RETICULATA, P. FRATERNUS P. TITHYMALOIDES*.

Plant	% Tannins	% Flavonoids	% Saponins	% Steroids and triterpenoids
K. reticulata (leaves)	6.00	26.68	12.04	34.48
P. fraternus (leaves)	2.82	16.20	2.31	16.40
Pedilanthus tithymaloides (flowers)	3.26	41.20	9.82	46.74

It was observed that the tannin and saponin contents were highest in the leaves of *K. reticulata* whereas, the flavonoid and steroid and triterpenoid in the flowers of *P. tithymaloides* **(Table 7)**. Khatoon *et al* (2006) reported very low content of tannins in the whole plant of *P. fraternus* (less than 0.2%) [41], however its fresh leaves in the present study showed 2.82% tannins.

Separation of extracted compounds by TLC:

Following results in Table 8 and 9 were obtained when the TLC of compounds was carried out.

TABLE 8: NUMBER OF BANDS OBSERVED IN THE TLC OF COMPOUNDS

Plant	No. of compounds (represented by bands) separated on TLC plates				
	Tannins	**Flavonoids**	**Saponins**	**Steroids**	**Triterpenoids**
K. reticulata	6	9	10	8	2
P. fraternus	8	8	8	11	1
P. tithymaloides	6	11	10	6	1

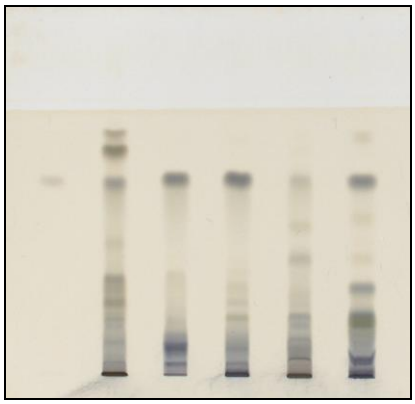

FIG.13: Separation of tannins by TLC

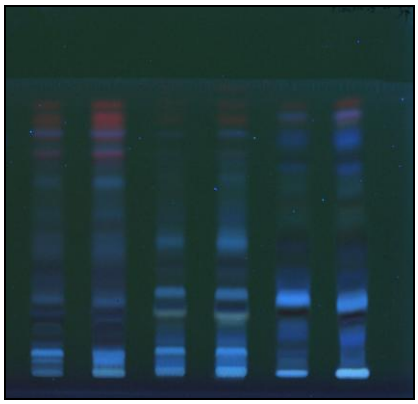

FIG.14: Separation of flavonoids by TLC

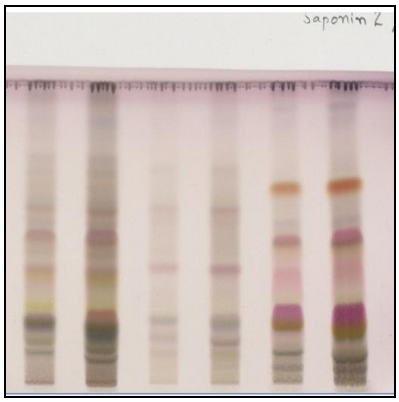

FIG.15: Separation of saponins by TLC

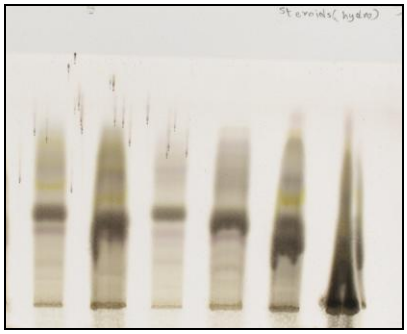

FIG. 16: Separation of steroids by TLC

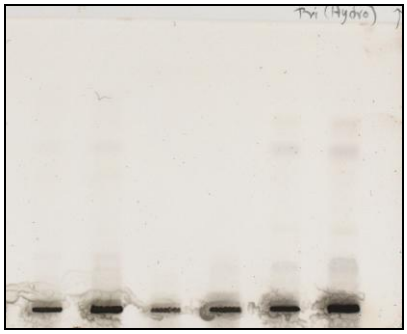

FIG. 17: Separation of triterpenoids by TLC

FIG. 13: Track 1 - Gallic acid std. Track 2 - *K. reticulata* leaves Track 3 - *P. fraternus* leaves Track 4 - *Pedilanthus tithymaloides* flowers]

FIG. 14, 15, 16 and **17:** Track 1 and 2 - *K. reticulata* leaves (2 and 5 µl respectively)
Track 3 and 4 - *P. fraternus* leaves (2 and 5 µl respectively)
Track 5 and 6 - *Pedilanthus tithymaloides* flowers (2 and 5 µl respectively)

TABLE 9: COMMON COMPONENTS IN ALL THE ALL THE EXTRACT ON TLC PLATES

TLC of compounds	Bands common in all the extracts
Tannins	4
Flavonoids	3
Saponins	2
Steroids	-
Triterpenoids	-

The leaves of *K. reticulata* and *P. fraternus* and flowers of of *P. tithymaloides* showed presence of 6, 8 and 6 components (bands) of tannins **(Fig.13), 9, 8** and **11** of flavonoids **(Fig.14)** and **10, 8** and **11** components of saponins (**Fig.15)**, **8, 11** and **6** of steroids **(Fig.16)** and **2, 1** and **1** bands of triterpenoids **(Fig.17)** respectively**(Table 8)**

Table 9 indicates that 4 components of tannins, 3 of flavonoids and 2 of saponins are present in all the extracts whereas TLC of steroids and triterpenoids did not show presence of any common

components **(Table 9)** (**Fig. 13** to **17).** Sivasankar *et al* (2011) reported presence of rutin in the leaves of *K. Reticulate* [42] however present study did not show rutin in the TLC of flavonoids.

CONCLUSIONS: Present study shows that the DMSO extracts of the leaves of *K. reticulata* and the flowers of *P. tithymaloides* inhibited the growth of *P. vulgaris* and *S. aureus* and their MIC values obtained were 200 mg /ml. The extracts did not inhibit the growth of *T. rubrum* and *T. mentagrophytes.* The phytochemical analysis of these extracts indicated that the tannins and saponins contents were highest in the leaves of *K. reticulata* whereas, the flavonoids and steroids and triterpenoids contents were highest in the flowers of *P. tithymaloides*. TLC of extracted tannins showed presence of 4 common components, the flavonoids showed 3 and the saponins showed 2 common components in these plants. TLC of steroids and triterpenoids showed no common components. The chemical compounds present in these plants should be isolated and tested for their antibacterial activity

ACKNOWLEDGEMENT: The authors gratefully acknowledge University Grants Commission for the financial assistance provided through the grant of Major Research Project to carry out this work. The authors express their sincere thanks to Principal, St. Xavier's College for providing the facilities. The authors are grateful to Dr. Medhekar, Head of Skin and V. D. Department, G.T. hospital and Dermatologist, Dr. Miskeen of Dr. Miskeen's Central Clinical Microbiology Laboratory, Thane for providing necessary facilities and help for the collection of dermatophyte samples.

REFERENCES:

1. Mohd AKR, Norhayati A H, Emynur SS, Mohamad FG, Azlina M., Tajul ZM, & Ahmad ZAL: Screening of seven types Terengganu herbs for their potential antibacterial activity against selected food microorganisms. Borneo Science 2012; 31:9-23.
2. http://apps.kew.org/wcsp/prepareChecklist.do;jsessionid=3C7211C9D96AE62A82EBC994626E1302?checklist=selected_families%40%40312081120131448266. World Checklist of Selected Plant Families, Kew Royal Botanic Gardens.
3. Bhagat R, Misar AV, Ambavade SD, and Kulkarni DK: HPTLC analysis and anti-inflammatory activity of *Jatropha gossypifolia* L. root in mice and Wistar rats. International Journal of Pharmacological Research 2013; 3(1):13-17.
4. Lai XZ, Yang YB, Luo X: The investigation of euphorbiaceous medicinal plants in southern China. Economic Botany 2004; 58(Supplement):S307-S320.
5. Shruthi SD, Ramachandra YL, Rai SP and Jha PK: Pharmacognostic evaluation of leaves of *Kirganelia reticulata* Baill.(Euphorbiaceae). The Asian and Australasian Journal of Plant Science and Biotechnology 2010; 4(1):62-65.
6. Singh B, Dutt N, Kumar D, Singh S and Mahajan R: Taxonomy, ethnobotany and antimicrobial activity of *Croton bonplandianum, Euphorbia hirta* and *Phyllanthus fraternus*. Journal of Advances in Developmental Research 2011; 2(1):21-29.
7. Jain SK and Srivastava S: Traditional uses of some Indian plants among islanders of Indian ocean. Indian Journal of Traditional Knowledge 2005; 4(4):345-357.
8. Seigler DS: Phytochemistry and Systematics of the Euphorbiaceae. Annals of the Missouri Botanical Garden 1994; 81(2):380-401.
9. Lam S, Wang C, Chen C, Lee S: Chemical Investigation of *Phyllanthus reticulatus* by HPLC-SPE-NMR and conventional Methods. Phytochem. Anal 2007; 18:251–255.
10. Abreu PM, Matthew S, Gonza T, Vanickova L, Costa D, Gomes A, Segundo MA, Fernandes: Isolation and identification of antioxidants from *Pedilanthus tithymaloides.* J Nat Med 2008; 62:67–70.
11. Auwal MS, Fatima AL, Shuaibu A, Ismaila AM, Kyari AS, Thaluvwa AB and Njobdi AB: Phytochemical properties and effect of aqueous extract of *Jatropha caucus* root bark on some bacterial isolates. Journal of Pharmacognosy and Phytotherapy 2013; 5(8):148-152.
12. Aneja KR: Experiments in Microbiology, Plant Pathology and Tissue Culture. Wishwa Prakashan, A division of Wiley Eastern Limited, 1993: 335-336.
13. Hamid AA, Aiyelaagbe OO, Ahmed RN, Usman LA and Adebayo SA: Preliminary phytochemistry, antibacterial and antifungal properties of extracts of *Asystasia gangetica* Linn T. Anderson grown in Nigeria. Advances in Applied Science Research 2011; 2(3):219-226.
14. Abubacker MN, Ramanathan R, Senthil Kumar T: *Invitro* antifungal activity of *Cassia alata* Linn. flower extract. Natural Product Radiance 2008; 7(1):6-9.
15. Webster D, Taschereau P, Rene BJ, Sand C, Rennie RP: Antifungal activity of medicinal plant extracts; preliminary screening studies. Journal of Ethnopharmacology 2008; 115(1):140-146.
16. Larone DH: Medically important fungi-a guide to identification. Harper and Row Publishers, Maryland, 1976: 83-84.
17. Kokate CK, Purohit AP, Gokhale SB: Pharmacognosy. Nirali Prakashan, Pune, Edition 39, 2007: 607-611.
18. Sadasivam S and Manickam A: Biochemical methods. New Age International (P) Ltd, New Delhi, Edition 3, 2008: 31-32.
19. Brindha S, Thamaraiselvi T, Maragathavalli S, Annadurai B and Gangwar SK: Studies on the chemical and medicinal value of *Vitex negundo* Linn. International Journal of Advanced Biological Research 2012; 2(2):298-301.
20. Edeoga HO, Okwu DE and Mbaebie BO: Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4(7):685-688.
21. Malu SP, Obochi GO, Edem CA and Nyong BE: Effect of methods of extraction on phytochemical constituents and antibacterial properties of Tetrecarpidium conophorum

seeds. Global Journal of Pure and applied Sciences 2009; 15(3):373-376.
22. Bai X., Qiu A. and Guan J: Optimization of microwave-assisted extraction of antihepatotoxic triterpenoid from *Actinidia deliciosa* root and its comparison with conventional extraction methods. Food Technol. Biotechnol 2007; 45(2):174-180.
23. Auribie MA: Antioxidant activity of tannin from *Tamarix aphylla L.* leaves. Basra J. Agric. Sci 2011; 24(1):1-10.
24. Wagner H, Bladt S, Zgainski EM: Plant drug analysis – A thin layer chromatography atlas. Springer, Verlag, 1984: 163-165 and 192.
25. Wagner H and Bladt S: Plant drug analysis-A thin layer chromatography atlas. Springer, Germany, Edition 2, 1996: 306.
26. Waksmundzka-Hajnos M, Sherma J and Kowalska T: Thin layer chromatography in phytochemistry. CRC Press, Taylor and Francis Group, Boca Raton, 2008: 581.
27. Schmitz D, Zapp J and Bernhardt R: Hydroxylation of the triterpenoid dipterocarpol with CYP106A2 from *Bacillus megaterium*. Federation of European Biochemical Societies Journal 2012; 279:1663–1674.
28. Bapat UC, Mhadnak TR and Mhapsekar DR: Antibacterial activity studies of extracts of *Phyllanthus fraternus* Webster, *Jatropha gossypifolia* Linn. and *Sapium sebiferum* (L.)Roxb. Xplore 2012; 3(1):1-9.
29. Eldeen IMS, Seowa E-M, Abdullaha R, Sulaimana SF: In vitro antibacterial, antioxidant, total phenolic contents and anti-HIV-1 reverse transcriptase activities of extracts of seven *Phyllanthus* sp. South African Journal of Botany 2011; 77:75–79.
30. Shruthi SD, Ramachandra YL, Rai PS, Shetty AV: Antibacterial Potential of leaf extracts from *Kirganelia reticulata* Baill. International Journal of Pharma Research and Development 2010; 2(6):1-7.
31. Sankannavar SH, Patil CG: *In vitro* studies on diversity of antibacterial activity in some species of *Phyllanthus* for human pathogenic bacteria. Asian J. Exp. Biol. Sci. 2012; 3(3):607-612.
32. Islam MS, Akhtar M M, Sharmin SA, Rahman MA, Rahman MM, Khalekuzzaman M, Anisuzzaman M and Alam MF: Antibacterial potential from leaf extracts from *Phyllanthus reticulatus* Poir. International Journal of Pharmaceutical Research and Development 2013; 5(4):88-95.
33. Adhikary S, Haldar PK, Kandar CC, Malakar P, Deb AR: Potential antimicrobial, antioxidant and wound healing properties of the different extract of *Pedilanthus tithymaloides*(L.) Poit. Leaves. International Journal of Phytopharmacology 2013; 4(3):204-211.
34. Chaudhari Y, Badhe M, Kumar EP, Mody H, Kokardekar R: An investigation of antibacterial activity of *Pedilanthus tithymaloides* on different strains of bacteria. Int. J. Pharm. Phytopharmacol. Res. 2012; 1(6):385-386.
35. Adejumo TO and Bamidele BS: Control of dermatophyte-causing agents (*Trichophyton mentagrophytes* and *Trichophyton rubrum*) using six medicinal plants. Journal of Medicinal Plants Research 2009; 3(11):906-913.
36. Narasimhudu CL and Raju RRV: Phytochemical constituents of *Phyllanthus* species (Euphorbiaceae) from easternghats of Andhra Pradesh, India. International Research Journal of Pharmacy 2012; 3(5):184-200.
37. Gopinath SM, Rakesh CK, Murthy TPN, Dayananda KS: Preliminary phytochemical evaluation of leaf extracts of *Gymnema sylvestre, Phyllanthus amarus, Phyllanthus reticulatus* of Siddarabetta, Tumkur district, Karnataka. International Journal of Pharmacognosy and Phytochemical Research 2012; 4(3):109-111.
38. Kantamreddi VSSN, Lakshmi YN and Kasapu VVVS: Preliminary phytochemical analysis of some important Indian plant species. The International Journal of Pharma and Bio Sciences 2010; 4(1):B351-358.
39. Raveen R, Dhayanidhi P, Dhinamala K, Arivoli S, Tennyson S: Larvicidal activity of *Pedilanthus tithymaloides* (L.) Poit (Euphorbiaceae) leaf against the dengue vector *Aedes aegypti* (Diptera: Culicidae). International Journal of Environmental Biology 2012; 2(2):36-40.
40. Prakash NKU, Bhuvaneswari S, Balamurugan A, Radhika B, Bhagya R, Sripriya N, Prameela L, Sarojini S, Vigneshwari R, Chandran M and Arokiyaraj S: Studies on phytochemistry of 100 plants in Chennai, India. British Journal of Pharmaceutical Research 2013; 3(3):407-419.
41. Khatoon S, Rai V, Rawat AKS, Mehrotra S. Comparative pharmacognostic studies of three *Phyllanthus* species. Journal of Ethnopharmacology 2006; 104:79–86.
42. Sivasankar V, Moorthi A, Sarathi Kannan D, Suganya devi P: Anthocyanin, and its an tioxidant properties in selected fruits. Journal of Pharmacy Research 2011; 4(3):800-806.

How to cite this article:
Bapat UC and Mhapsekar DR: Study of Antimicrobial Activity and Phytochemical Evaluation of *Jatropha Gossypifolia*, *Sapium Sebiferum*, *Kirganelia Reticulata*, *Phyllanthus Fraternus* and *Pedilanthus Tithymaloides* . Int J Pharm Sci Res 2014; 5(11): 4933-41.doi: 10.13040/IJPSR.0975-8232.5 (11).4933-41.