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THERAPEUTIC POTENTIAL OF BIOACTIVE PHYTOCHEMICALS BY INHIBITING β-LACTAMASE OF MULTIDRUG RESISTANT CLINICAL ISOLATES

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Keywords:

β-lactam-resistant bacteria, antimicrobial activity, Medicinal plants, phytochemicals, β-lactamase (βL) , nitrocefin

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ABSTRACT: β-lactams have been widely used as antibiotics for treatment of nosocomial and community acquired infections for the last five decades. Under selective pressure from the extensive use of cephalosporins in 1980s and 1990s, many bacteria have emerged as resistant against these antibiotics. The resistance to β-lactam antibiotics can be due to any of the following three mechanisms i.e. decreased accumulation of the drugs by bacterial cell, hydrolysis of the antibiotics by β-lactamases (βL) and alterations in penicillin binding proteins that reduce their affinity for the drug. WHO has repeatedly warned for a growing emergence of bacterial antibiotic resistance. The consequences of drug resistance include higher mortality and morbidity. Hence there is a need to search for new alternative antimicrobial agents with fewer side effects. In the current study, we have checked the antimicrobial and antioxidant activities of ten traditionally used medicinal plants against β-lactam resistant bacteria isolated from urinary tract infected (UTI) patients. We have also proposed phytochemicals extracted from medicinal plants as potential antibacterials and investigation of inhibition of βL activity. The enzymes were purified and studied for inhibition assay by using nitrocefin as a substrate.

INTRODUCTION: The use of plant or its part in treatment of bacterial diseases has been an ancient practice and is an important component of healthcare system in India. Bacteria have the hereditary potential to acquire resistance to drugs. Antibiotic resistance is the major problem in the treatment of in- and out- patients [1]. The World Health Organisation (WHO) estimated that 80% population of Asian and African countries presently use herbal medicine for primary healthcare.

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Considerable research has been carried out on Pharmacognosy, chemistry, pharmacology and clinical therapeutics of Ayurvedic medicinal plants. Various studies and research is underway to investigate the antimicrobial potency of medicinal plants. Many reports have showed the effectiveness of traditional herbs against microorganisms.

The β-lactams constitute one of the most important antibiotic families in worldwide use. But extensive use of antibiotics has evolved bacteria resistant to antibiotics. From the late 1990s, multidrug-resistant Enterobacteriaceae (mostly *Escherichia coli*) produced extended-spectrum βL (ESBLs) [2]. The incidence of antibiotic resistance among ESBL-producing *Escherichia coli* has increased in recent years. ESBLs appear mainly due to mutations in βL encoded by the SHV, TEM, and CTX-M genes [3, 4].

Due to consequences of drug resistance bacteria, there is an urgent need to develop an antimicrobial agent with lesser side effects and increased potency to inhibit multidrug resistant bacteria. Hence, the antimicrobial activities of plant extracts were evaluated against clinically proved β-lactam-resistant bacteria (*Escherichia coli, Pseudomonas aeruginosa and Enterococcus faecalis*) and reference strains of bacteria (*Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212) by using disc-diffusion assay.

MATERIALS AND METHODS:

Test microorganisms:

The bacteria were isolated from urine samples of urinary tract infected patients. These samples were collected in a sterile container from pathology laboratories in Nagpur, Maharashtra, India. The bacterial colonies were isolated by streaking each urine sample on LB agar plate (Himedia DT001) and then identified by Gram's staining and biochemical tests (Himedia KBM001, KB002, KB003) [5]. The antibiotic sensitivity was done by Kirby-Bauer method [6]. The resultant MDR *E. coli* strains were selected for isolating β-lactam resistant strains and the ESBL detection was done with following antibiotics: Cefazolin, Cefaclor, Cefixime, Cefepime (Himedia FD278).

Plant material:

The fresh leaves of 10 plant species used in traditional medicines *[Andrographis paniculata (Ap), Astercantha longifolia (Al), Bixa orellana (Bo), Gardenia resinifera (Gr), Pongamia pinnata (Pp), Psoralea corylifolia (Pc), Sphaeranthus indicus (Si), Solanum trilobatum (St), Soyamida febrifuga (Sf)* and *Thespesia populnea (Tp)]* were collected from Nagpur region, MS, India in 2013 **(Table 1**). All plants were identified by a taxonomist at the Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur.

TABLE 1: DETAILS OF SELECTED TEN MEDICINAL PLANTS:

Family	Botanical name	Local name	Voucher number	Uses in traditional medicine
Acanthaceae	*Andrograpis paniculata* (Burm.f.)Wall. Ex Nees	Bhuinimb, Kalmegh	9038	Diarrhea, leprosy, pneumonia, tuberculosis, gonorrhoea, syphilis, malaria, cholera [7]
Acanthaceae	*Astercantha longifolia* (L.) Nees	Kokilaksha, Talamkhana	9039	anti-inflammatory, antitumor, antidysentric, antibacterial [8]
Bixaceae	*Bixa orellana* L.	Annatto, Latkan	9041	gonorrhoea, dysentery and hepatitis [9]
Rubiaceae	*Gardenia resinifera Roth.*	*Periakambi, Dikemadi,*	10012	astringent to bowels, relieves pain of bronchitis, vomiting and constipation [10]
Fabaceae	*Pongamia pinnata* (L.) Pierre	Karanj	10037	Rheumatism, piles, female genital tract infection, ulcers and haemorrhoids [11]
Fabaceae	*Psoralea corylifolia* L.	Babchi	10038	Vitiligo and other skin problems [12]
Acanthaceae	*Sphaeranthus indicus* Linnaeus	Gorakhmundi	10039	immunomodulatory, antioxidant, anti-inflammatory, bronchodialatory, hepatoprotective [13]
Solanaceae	*Solanum trilobatum* L.	Mothiringni, Kateri, Kantkari	10041	skin diseases, hemeplegia, edema, urinary calculi, amenorrhea, and urinary tract disorders [14]
Meliaceae	*Soymida febrifuga* (Roxb.) Juss	Raktarohan	10042	vaginal infections, dental diseases, rheumatic pains and stomach pains [15]
Malvaceae	*Thespesia populnea* (L.)Sol.ex Correa	Paraspimpal	10043	antifertility, antimicrobial, antiinflammatory, antioxidant, purgative and hepatoprotective activity [16]

Preparation of extract:

The fresh leaves of all plants were washed with water, shade dried and powdered. 30g of leaf powder was used for phytochemical extraction through soxhlet apparatus using successive 300ml of Petroleum ether (60-80 ^{0}C), acetone, chloroform (61^{0}C), methanol (78.5^{0}C) and water (80^{0}C). The solvents were selected according to their polarity. The extracts obtained were kept for solvent evaporation and stored in sealed tubes at 4 ^{0}C.

Alternatively, cold maceration of the coarsely powdered leaves of all plants were carried out by soaking 30g of the powder in 150ml of 50% aqueous methanol with continuous shaking on rotary shaker for ten days. The filtrate was then evaporated to 30ml. The extracts obtained by Soxhletion and cold maceration were dissolved in distilled water and used for qualitative, quantitative phytochemical estimation and antibacterial analysis.

Phytochemical testing:

The plant extracts obtained by Soxhletion with five subsequent solvents of increasing polarity and by cold extraction were assessed for qualitative phytochemical profiling. On comparision, it was found that cold extracts gave better results than hot extracts and therefore the extracts obtained upon cold extraction were used for quantitative estimation of phytochemicals.

Phytochemicals identification by HPLC:

The methanolic extracts obtained by cold maceration have shown significant qualitative and quantitative yield, hence were used for HPLC analysis. HPLC was performed by Reverse phase C-18-aminopack zorbax eclipse-AAA column with SPD 10 AVP pump. Methanol: water (90:10 v/v) was utilized as mobile phase. The class VP integration software was used for data analysis.

Antioxidant activity

The antioxidant activity of all extracts obtained by hot and cold extraction methods were investigated by FRAP assay [17-20]. 25µg extract in 1 ml of distilled water with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of potassium ferricyanide were taken and incubated at 50°C for 30 min. 2.5 ml of trichloroacetic acid were added and centrifuged at 3000 rpm for 10 min. 2.5 ml of upper layer was pipette out. To it, 2.5 ml of distilled water was added along with 0.5 ml $FeCl_3$. The absorbance was read at 700nm. Ascorbic acid was used as a standard [21-24].

Antimicrobial assays:

The antibacterial activity of each solvent extract was measured *in vitro* against 15 clinical MDR (multidrug resistant) isolates representing five of *E. coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* each. The antibacterial potential was investigated by disc diffusion method, as recommended by CLSI. Single colony from the UTI agar plate was inoculated in 10ml of LB media and incubated at 37^0C for 14-16 hrs to match McFarland's turbidity standard of 0.5 which was equal to 1.5×10^8 cells/ml. This culture was then used for antibiotic susceptibility testing by disc diffusion method for antibacterial assay by Bauer-Kirby method [22].

The plates were examined for zone of inhibition and recorded as sensitive, intermediate and resistant referring zone size interpretive chart (Himedia).

Minimum inhibitory concentration (MIC):

25ml molten Muller-Hilton agar was inoculated with 10µl of each clinical isolate matched with 0.5 McFarland's turbidity standard and poured in sterile petri dishes. After complete solidification of agar, the discs of 1mg-10mg of plant extract obtained through cold maceration were placed on top of the plate. These petriplates were incubated at 37^oC for 18 hours. The result was observed for inhibition of bacterial growth.

Antibacterial activity of phytochemicals:

The phytochemicals identified by HPLC were investigated to check the antibacterial activity. Phytochemicals showing significant antibacterial activity were further analysed for their inhibitory activity against β-lactamase.

Phytochemicals inhibiting the β-lactamase activity:

- **Production and purification of crude β-lactamase enzyme:**

Single colony of *E. coli* culture was grown in nutrient broth containing ampicillin (20µg/ml) as an inducer for enzyme production. This was harvested by centrifugation (7,000 x g, 20 min at 4°C), and washed twice in phosphate buffer (0.01 M, pH 7.0) at 4°C. The extracellular medium was used for purification of β-lactamase.

- **Ammonium sulphate precipitation:**

Powdered ammonium sulphate AR was added up to 80% saturation. The crude enzyme obtained was brought to 60 % saturation with ammonium

sulphate at pH 8 and kept overnight at 4°C. After equilibration, the supernatant was brought to 80 % saturation with ammonium sulphate and centrifuged at 8000 rpm, at 4^0C for 10 min. Then the precipitates were collected separately and dissolved in a 0.1 M phosphate buffer at pH 8 and stored at 4^0C for further purification [25].

- **Dialysis:**

The pre-treated dialysis membranes (LA395, Himedia) were used for dialysis of the precipitates collected after ammonium sulphate precipitation. The precipitate was dissolved in 0.1M phosphate buffer (pH 8) and dialyzed. After dialysis, the samples were used for protein estimation and enzyme assay [25].

- **Desalting:**

The dialyzed enzyme (2ml) was applied to Sephadex G-25 column that was pre- equilibrated with 0.1 M Phosphate buffer (pH 8). The protein elution was done with the same buffer. The fractions were collected and assayed for protein at 280 nm as well as for enzyme activity with nitrocefin. The active fractions were pooled, dialyzed against the 0.1 M phosphate buffer at pH 8 and concentrated to yield 9.87 mU/mg protein [25].

- **Inhibition of β-lactamase activity assay:**

Nitrocefin is a chromogenic cephalosporin substrate of βLs. As a cephalosporin, nitrocefin contains a β-lactam ring which is susceptible to β-lactamase mediated hydrolysis. Hydrolysis of nitrocefin produces a shift of ultraviolet absorption inside the visible light spectrum from intact (yellow) nitrocefin (~380 nm) to degraded (red) nitrocefin (~490 nm) allowing visual detection of β-lactamase activity [26]. The enzyme was incubated with 10µM of phytochemicals (Chlorogenic acid, naringenin, quercetin, salicylic acid and theophylline) and the βL activity was checked. Significant inhibition of the enzyme activity was noted. The activity was calculated as:

βL activity = B/(ΔT*V)*D = mU/mg of protein

where, B – Amount of nitrocefin (nmol), ΔT – reaction time (min), V – Sample volume (ml)
D – sample dilution factor

RESULTS AND DISCUSSION:

Isolation and identification of clinical isolates:

The bacteria identified were *Escherichia coli* ***(26%),*** *Enterococcus faecalis* (35%), *and Pseudomonas aeruginosa* (36%) out of total 100 selected MDR clinical isolates [5-6]. Below mentioned **Table 2** denotes qualitative phytochemical analysis. All the selected ten plants showed presence of sterols,, alkaloids, saponins, Glycosides, tannins and phenols. Absence of anthroquinones in Al, Bo, Gr, Pc, St, Si and Tp was observed.

TABLE 2: QUALITATIVE PHYTOCHEMICAL ANALYSIS

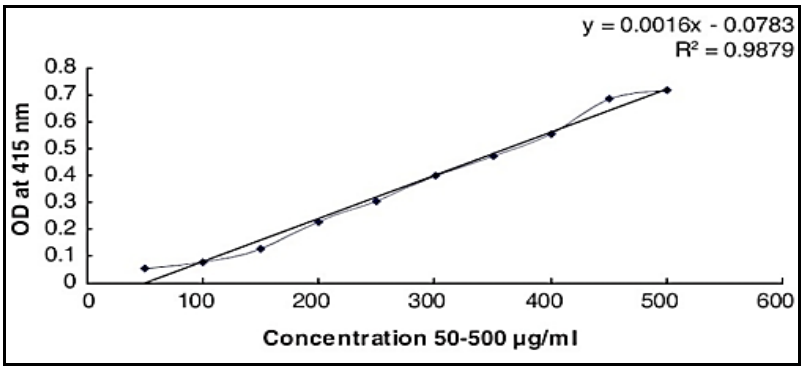
Plants	Phytochemicals						
	Sterols	Alkaloids	Saponins	Glycosides	Anthroquinones	Tannins	Phenols
Ap	+	+	+	+	+	+	+
Al	+	+	+	+	-	+	+
Bo	+	+	+	+	-	+	+
Gr	+	+	+	+	-	+	+
Pp	+	+	+	+	+	+	+
Pc	+	+	+	+	-	+	+
St	+	+	+	+	-	+	+
Sf	+	+	+	+	+	+	+
Si	+	+	+	+	-	+	+
Tp	+	+	+	+	-	+	+

Upon quantitative analysis of phytochemicals (**Table 3**), highest concentration of alkaloids in Tp, saponin in Al, flavonods in Gr, phenol in Bo, tannins in Bo and antocyanidin in Gr was observed.

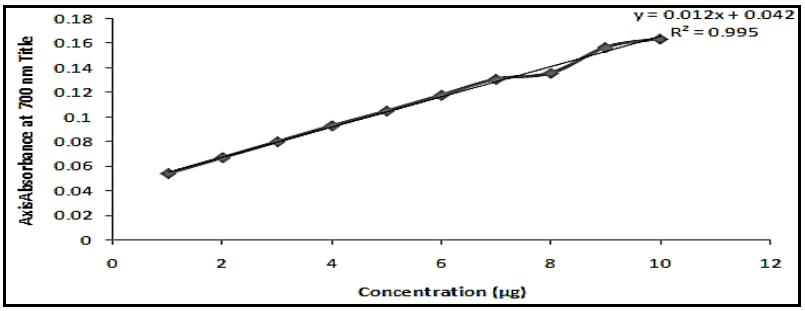
The following four graphs describe the standard curves for quercetin, gallic acid, tannic acid and catechin for quantitative estimation of flavanoids, phenol, tannin and anthocyanidine estimation.

Plant Name	% yield of phytochemicals					
	Alkaloids	Saponin	Flavonoids	Phenol	Tannin	Anthocyanidin
Ap	5.00	1.168	0.54	0.0157	0.25899	0.16992
Al	0.48	2.948	0.94	0.0097	0.12408	0.31284
Bo	4.89	1.004	1.31	0.1703	1.08419	0.17064
Gr	4.65	1.746	2.36	0.028	0.22058	0.63936
Pp	1.92	0.959	0.77	0.031	0.17578	0.12936
Pc	1.20	1.684	0.68	0.015	0.13786	0.15689
St	3.06	0.365	1.73	0.018	0.192	0.1376
Sf	4.82	1.153	0.932	0.013	0.12408	0.05388
Si	0.20	0.304	0.65	0.017	0.310	0.0608
Tp	15.74	0.875	0.63	0.076	0.5611	0.05388

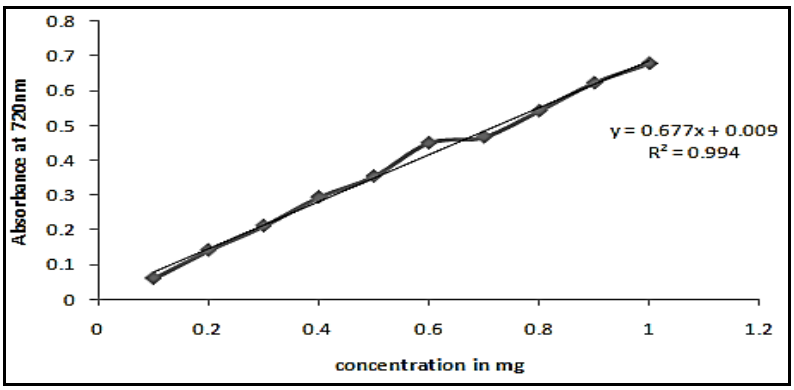
TABLE 3: QUANTITATIVE PHYTOCHEMICAL ANALYSIS



GRAPH 1: STANDARD QUERCETIN CURVE FOR FLAVANOID ESTIMATION [21]
[Flavanoid concentration as quercetin equivalent per gram of plant material]

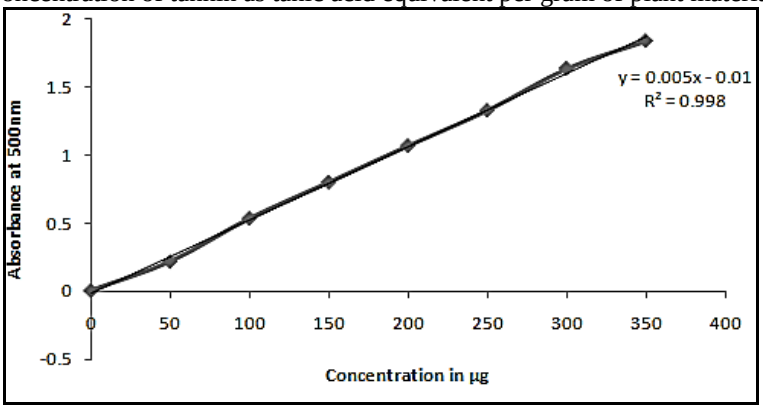


GRAPH 2: STANDARD GALLIC ACID CURVE FOR PHENOL ESTIMATION
Concentration of total phenol as gallic acid equivalent per gram of plant material



GRAPH 3: STANDARD TANNIC ACID CURVE FOR TANNIN ESTIMATION

Concentration of tannin as tanic acid equivalent per gram of plant material



GRAPH 4: STANDARD CATECHIN CURVE FOR ANTHOCYANIDINE ESTIMATION

Concentration of anthocyanidin per catechin equivalent per gram of plant material

Identification of phytochemicals by HPLC:

The HPLC chromatograms reveal the presence of tannic acid, ellagic acid, quercetin, chlorogenic acid, naringenin, theophylline, betulinic acid, resorcinol, catechol, salicylic acid, vanillin, gallic acid, squalene and pyrogallol in selected plants as described in **Table 4**.

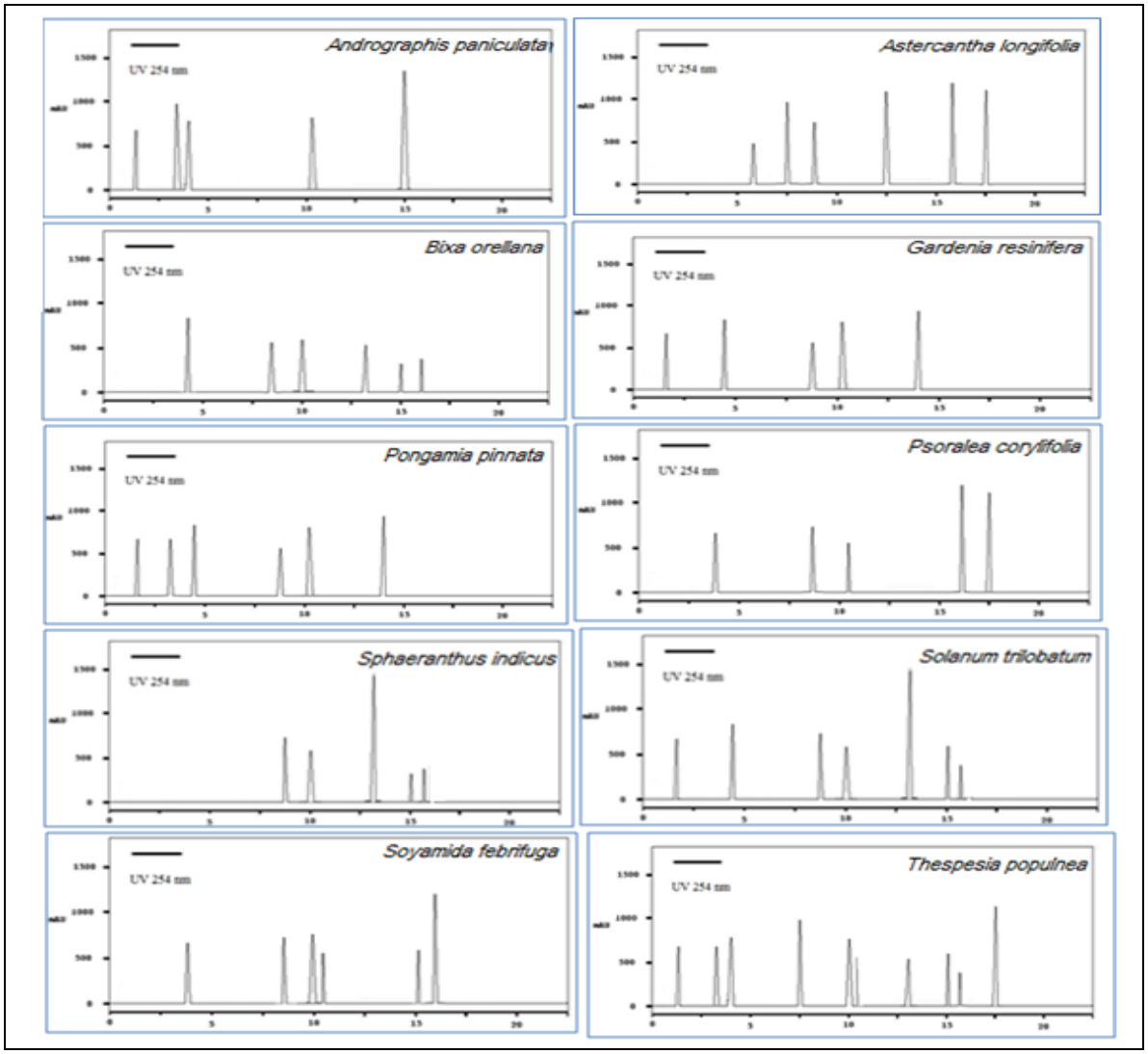


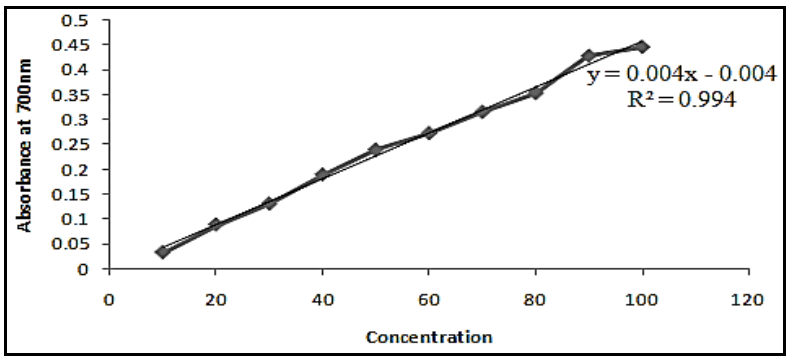
FIG.1: HPLC CHROMATOGRAMS OF PHYTOCHEMICALS FROM AFOREMENTIONED PLANTS

TABLE 4: PHYTOCHEMICALS IDENTIFIED BY HPLC

Sr. No.	Retention Time in Min	Peak Area	Phytochemical	Plants name
1	1.25	625	Tannic acid	*Ap, Gr, Pp, St,Tp*
2	4.44	854	Ellagic acid	*Ap, Pp, St,Tp*
3	3.02	958	Quercetin	*Ap, Pc, Sf,*
4	10.02	758	Chlorogenic acid	*Ap, Bo, Gr, Pp, Si, St, Sf, Tp*
5	15.00	1235	2-Furaldehyde,5 (hydroxy methyl)	*Ap, Bo, Si, St, Sf, Tp*
6	3.89	558	Naringenin	*Pp, Tp*
7	10.98	501	Theophylline	*Pc,Sf, Tp*
8	5.82	425	Betulinic acid	*Al*
9	12.50	1001	Resorcinol	*Al*
10	7.48	1001	Catechol	*Al, Tp*
11	17.50	1123	Salicylic acid	*Al, Pc,Sf, Tp*
12	8.54	625	Vanillin	*Al, Pp,Sf*
13	16.23	1234	Hexadecanoic acid	*Al, Bo, Pc, Si, St, Tp*
14	13.02	526	3-o-methyl glucose	*Bo, Si, St, Tp*
15	4.62	802	Gallic acid	*Bo, Gr*
16	8.95	596	Squalene	*Bo, Gr, Pc, Si, St*
17	14.25	977	Pyrogallol	*Gr, Pp*

Antioxidant activity:

The highest antioxidant activity was observed in water extract compared to extracts of organic solvents. The antioxidant activity per ascorbic acid equivalent is mentioned in **Table 5**, for all the selected ten plants. Highest activity is seen in acetone extract of *Bixa orellana* while lowest recorded antioxidant activity was possessed by petroleum ether extract of *Pongamia pinnata.*



STANDARD ASCORBIC ACID CURVE FOR ANTIOXIDANT ASSAY

TABLE 5: ANTIOXIDANT ACTIVITY PER ASCORBIC ACID EQUIVALENT

Sr. No.	Plant	Concentration (µg)				
		PE	C	A	M	Aq
1	*Andrographis paniculata*	38.75	37.00	45.00	71.75	333.75
2	*Astercantha longifolia*	52.75	185.75	1331.25	1234.5	1199.25
3	*Bixa orellana*	37.5	243.5	1396.5	1264.5	1264.5
4	*Pongamia pinnata*	10.00	46.75	492.75	327.75	1112.25
5	*Psoralea corylifolia*	115.25	147.25	289.75	80.75	361.00
6	*Thespesia populnea*	126.25	472.25	1099.5	1145.25	1225.5
7	*Gardenia resinfera*	20.5	49.79	91.00	465.75	786.00
8	*Solanum trilobatum*	11.5	253.75	454.5	438.5	310.25
9	*Sphaeranthus indica*	100.0	127.5	75.00	960.00	107.5
10	*Soyamida febrifuga*	37.7	123.6	87.54	764.1	65.88

Antibacterial activity: Based on the phytochemical analysis of plant extracts, it was found that the methanolic extract has significant quantity of phytochemicals. Hence, methanolic extracts were selected for antibacterial activity against the MDR strains isolated. The cold

methanolic extracts have shown significant antibacterial activity as compared to soxhlet methanolic extract. Since all plants showed commendable antibacterial activity, it was planned to study the Minimum Inhibitory Concentration (MIC) for all the plants. **Table 6** represents the MIC observations.

TABLE 6: MIC OF PLANT EXTRACTS AGAINST MDR CLINICAL ISOLATES

S.No.	Plant extract	Microorganisms used		
		E.coli	***E. faecalis***	***P. aeruginosa***
1	*Ap*	2 mg	1 mg	3 mg
2	*Al*	2 mg	3 mg	1 mg
3	*Bo*	6 mg	6 mg	8 mg
4	*Gr*	2 mg	8 mg	4 mg
5	*Pp*	2 mg	2 mg	4 mg
6	*Pc*	2 mg	2 mg	4 mg
7	*St*	4 mg	0.4 mg	2 mg
8	*Sf*	6 mg	4 mg	2 mg
9	*Si*	4 mg	2 mg	1 mg
10	*Tp*	2 mg	2 mg	4 mg

Since, the selected plants have shown admirable antibacterial activity against uropathogenic MDR microorganisms and also the MIC results are promising, it was justified to study the antibacterial effect of pure phytochemicals against uropathogenic MDR *E. coli*, *E. faecalis* and *P. aeruginosa.* **Table 7** denotes this data.

TABLE 7: PURE PHYTOCHEMICALS USED TO CHECK ANTIBACTERIAL ACTIVITY

S.No.	Phytochemical (1mg)	Antibacterial activity (Inhibition zone, mm)		
		E.coli	***E. faecalis***	***P. aeruginosa***
1.	Betulinic acid	-----	-----	-----
2.	Catechol	17	12	14
3.	Chlorogenic acid	11	10	11
4.	Ellagic acid	10	11	12
5.	Gallic acid	15	17	13
6.	Naringenin	12	11	10
7.	Pyrogallol	15	14	12
8.	Quercetin	10	11	13
9.	Resorcinol	<10	10	11
10.	Salicylic acid	<10	11	10
11.	Squalene	-----	-----	-----
12.	Tannic acid	17	13	12
13.	Theophylline	10	<10	<10
14.	Vanillin	10	11	10

From the above observations, it is evident that catechol, gallic acid, pyrogallol and tannic acid act as very good antibacterial agents. **Table 8:** represents the specific activity of β-lactamase enzyme during various stages of purification.

TABLE 8: SUMMARY OF ENZYME PURIFICATION

S.No.	Purification step	Specific activity (Nitrocefin assay)
1	Crude extract	4.19
2	$(NH_4)_2SO_4$ precipitation	7.58
3	After dialysis	7.99
4	After desalting	9.87

Following image shows the result of βL analysed through SDS-PAGE. The expected protein band for βL is 30KDa. A single band of 30KDa was obtained upon desalting of protein.

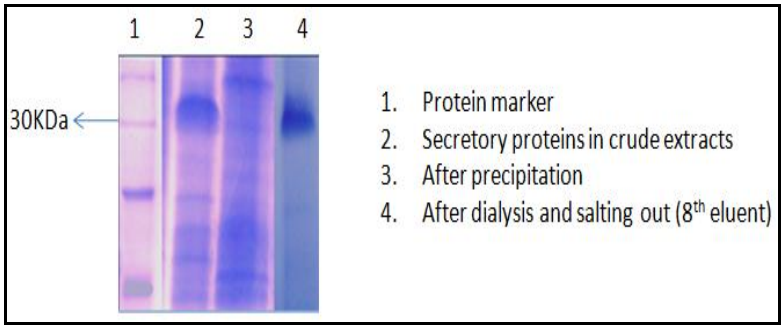


FIG.2: 10% SDS-PAGE

Following **Table 9** reports the βL activity in presence and absence of pure phytochemicals. The results showed that chlorogenic acid, naringenin, quercetin, salicylic acid, tannic acid and theophyllin could be the potential βL inhibitors.

TABLE 9: PHYTOCHEMICALS USED TO CHECK BL INHIBITION ACTIVITY

S.No.	Phytochemical	βL activity in absence of phytochemical	βL activity in presence of phytochemical
1.	Catechol	0.8867	0.6523
2.	Chlorogenic acid	0.8961	-0.2122
3.	Ellagic acid	0.7865	0.6854
4.	Gallic acid	0.8721	0.5495
5.	Naringenin	0.9862	0.0096
6.	Pyrogallol	0.9137	0.8806
7.	Quercetin	0.7589	-0.2311
8.	Resorcinol	0.6529	0.4321
9.	Salicylic acid	0.8952	0.0043
10.	Tannic acid	0.9821	-0.2378
11.	Theophylline	0.7635	0.0008
12.	Vanillin	0.5791	0.3218

DISCUSSION: The urinary tract infection (UTI) is serious bacterial infection that damages kidneys if untreated and is fatal. Most bacteria show resistance toward commonly used antibiotics. Hence, there is an urgent need to develop a good drug therapy regimen accordingly for recurrent and difficult to treat MDR UTI. The main mechanisms bacteria use to develop resistance against β-lactam antibiotics are the synthesis and secretion of βL that destroys the β-lactam ring of the antibiotic. The phytochemicals from *Andrographis paniculata (Ap), Astercantha longifolia (Al), Bixa orellana (Bo), Gardenia resinifera (Gr), Pongamia pinnata (Pp), Psoralea corylifolia (Pc), Sphaeranthus indicus (Si), Solanum trilobatum (St), Soyamida febrifuga (Sf)* and *Thespesia populnea (Tp)* have shown promising antibacterial activity and it is evident that they inhibit β-lactamase activity. The secretory βL enzyme were purified and investigated for its activity by nitrocefin as a substrate with and without aforementioned phytochemicals. This proves that chlorogenic acid, naringenin, quercetin, salicylic acid and theophylline have shown significant βL inhibitory activity.

Though chemically available chlorogenic acid, naringenin, quercetin, salicylic acid and theophylline do show antibacterial activity and significant βL inhibitory activity, they cannot be directly used for the treatment of MDR UTI. Hence, it is suggested that aforementioned plants which have shown the presence of chlorogenic acid, naringenin, quercetin, salicylic acid and theophylline can be successfully implemented in the treatment of difficult to treat recurring MDR UTI with less or no side effects. Also, these mentioned medicinal plants are low cost and are easily available.

Further studies on toxicity profiling of these medicinal plants can be undertaken along with ADME testing to clearly decide upon the dosage

for treatment against MDR UTI infections employing these medicinal plants.

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