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Research Article

ISOLATION OF BIOACTIVE COMPOUNDS AND BIOEFFICACIES OF *PERGULARIA DAEMIA* (FORSK.) CHIOV. CELL CULTURES

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ABSTRACT
Pergularia daemia (Forsk.) Chiov., a perennial twining herb grows wildly in South Asia and Africa continents, known for its anti-inflammatory, antipyretic, analgesic, antifertility, antidiabetic and hepatoprotective properties. In the present study, cell cultures of leaf explants were established on Murashige and Skoogs's medium (MS; 1962) supplemented with 2,4-D (2 mg l^{-1}) and kinetin (0.5 mg l^{-1}). Callus was harvested at the transfer age of 2, 4, 6, 8 and 10 weeks, dried, extracted in ethanol for the estimation of total phenolic, flavonoids, antimicrobial and antioxidant efficacies. In phytochemical studies α-, β-amyrin, β-sitosterol, lupeol, lupeol acetate, oleanolic acid, kaempferol, quercetin were isolated and quantified. Higher levels of phenolics and flavonoids were observed in 4 weeks-old callus (12.86 ± 0.61 mg GAE g^{-1} dw and 17.1 ± 0.10 mg QE g^{-1} dw respectively) with lower IC_{50} values (0.060); likewise, 4 weeks-old callus extract exhibited higher FRAP levels (336.66 ± 2.14 mg AAE g^{-1} dw). In antibacterial screening, 6 weeks-old callus demonstrated maximum inhibition against *B. subtilis*, *E. aerogenes* and *S. aureus* (IZ 12.66 ± 0.57, 12.33 ± 0.57 and 12.00 ± 0.00 mm respectively).

Keywords*: Pergularia daemia, Phytochemicals evaluation, Antimicrobial, Antioxidant efficacies*

INTRODUCTION
Reactive oxygen species are generated as byproducts of normal metabolism (Wang and Jiao, 2000). Medicinal plant-derived antioxidants such as terpenoids and phenolics received growing attention because they are known as chemopreventive agents against oxidative damage. Triterpenoids and phenolic compounds are naturally distributed in plants proved effective and powerful reducing agents (Schanderl, 1970; Chung, 1998). These natural compounds help in decreasing the heart disease (Williams and Elliot 1997) with antiinflammatory and anticarcinogenic properties (Miyake et al., 1999). Polyphenols are considered to be plant chemical defenses against pathogens and herbivores (Bernays *et al.*, 1989). The resistance to antimicrobial agents has become an increasingly important and pressing global problem (Cushnie and Lamb, 2005). Microorganisms have unfavorable effects on human health. Synthetic antioxidants are widely used in the food and health fields; however, in several cases they possess toxic and carcinogenic effects. Thereby, the interest in finding natural antioxidants, without undesirable side effects, has greatly increased (Baydar *et al.*, 2004).

Genus *Pergularia* is widely distributed in the old world tropics and subtropics from southern and tropical Africa and Asia, having multiple applications in different folk medicine, including the Indian system of *Ayurved*. *Pergularia daemia* (Forsk.) Chiov. (Asclepiadaceae) is a foetid smelling laticiferous twiner found in the plains throughout the hot parts of India. Plant used to cure eye sore, rheumatism, edema, kidney pains; infantile diarrhea, antihelmenthic, laxative, antipyretic, jaundice, asthma and rumen swelling (Kerharo and Bouquet, 1950; Nayar *et al.*, 1956; Kerharo and Adam, 1974; Kirtikar and Basu, 1983). Phytochemical review revealed presence of triterpenes, saponins, cardenolides, alkaloids and steroidal compounds (Anajanyulu *et al.*, 1998; Jain et al. 1998). Pharmacologically antiinflammatory, antipyretic, analgesic (Jain *et al.*, 1998), antifertility (Sadik *et al.*, 2001), antidiabetic (Wahi *et al.*, 2002),

antibacterial (Senthilkumar *et al.*, 2005) and hepatoprotective activities (Sureshkumar and Mishra, 2007) have been demonstrated.

Therefore, the present research work *in vitro* phytochemical estimation of *P. daemia* cell cultures as well their pharmacological activities were estimated and compared with *in vivo* plant.

MATERIALS AND METHODS

Plant material

The plant material was collected from the Campus, University of Kota, Kota, India, during the period of January-February 2018, authenticated from the Herbarium, Department of Botany, University of Kota, Kota and voucher specimen has been deposited (Herbarium Sheet No. 20147).

Tissue culture

Leaf explants from 4 months-old plant were collected and the excised explants washed under running tap water followed with 2% commercial grade detergent (Tween 20; 5 min). Surface sterilization was done by keeping in mercuric chloride (0.1% for 4 min) and thoroughly rinsed with sterile distilled water. Sterilized pieces (1 inch) were cultured on Murashige and Skoog medium (MS; Murashige and Skoog's 1962) consisting of basal salts, vitamins, 3% (w/v) sucrose and 0.8% agar with 2,4-dichlorophenoxyacetic acid (2,4-D; 2 mg l^{-1}) and kinetin (kn; 0.5 mg l^{-1}). The pH of the medium was adjusted to 6.8 and autoclaved at 15 psi for 15 min. Cultures were maintained at 26±2^{o}C under 16 hours photoperiod illuminated by fluorescent light (2000-3000 lux) and 55±5% relative humidity.

The callus was maintained for a period of 6 months by frequent subculturings (6-8 weeks interval) on to similar medium. Callus growth was evaluated in terms of fresh weight after 30 days of culture. The callus was harvested at the transfer age of 2, 4, 6, 8 and 10 weeks, kept at 100°C (for 5 minutes) so as to inactivate the enzymatic activities later, at 60°C, till a constant weight achieved.

Growth indices (GI) were calculated on dry weight basis by examining five replicates, and mean value as also the moisture content (%) was calculated. Later, the callus was dried and finely powdered for phytochemical and biological studies.

Extract preparation

For preparation of ethanolic extracts, 6 weeks-old whole plant (including leaves, roots, stem and flowers) was harvested, shade-dried and powdered. Similarly, callus at 2, 4, 6 and 8 weeks was harvested weighed, their moisture and growth indices were calculated. 100 g of plant material and 20 g of dried callus was extracted in ethanol (3×18 hr), filtered through Whatman filter paper No. 1, filtrate evaporated to dryness *in vacuo* and stored at 4^{o}C, until used.

Total phenolic and flavonoid contents

The total phenolics content was determined with Folin-Ciocalteau reagent (Bray and Thorpe, 1954). Optical density (OD) was measured at 750 nm. A standard calibration curve of gallic acid (10-500 mg ml^{-1}) was prepared and total phenolics in the extracts were expressed in mg of gallic acid equivalents (mg GAE g^{-1}) of extract. Total flavonoids were estimated by $AlCl_3$ spectrophotometic method (Zhishen *et al.*, 1999). A standard curve of quercetin (10-100 mg ml^{-1}) was prepared. The total flavonoids were expressed as mg of quercetin equivalents (mg QE g^{-1}) of extract.

Isolation of compounds

For the isolation of triterpenoid, ethanolic extracts and for flavonoid glycosides acid-hydrolyzed extract (7% H_2SO_4; 10 ml g^{-1} for 2 h; hydrolysates were filtered, extracted with ethyl acetate, washed with DW till neutrality) were applied on TLC plates, developed in selected organic solvent systems (Table 1). Developed plates were air-dried, sprayed with 10% alcoholic H_2SO_4, anisaldehyde reagents for triterpenoids and 5% alcoholic $FeCl_3$ as also with 1% alcoholic $AlCl_3$ separately for flavonoids and heated to 100^{o}C for few min, until characteristic colors developed and their Rf values calculated.

Preparative TLC was performed on activated silica gel plates which were developed in benzene-haptane-alcohol (100:100:1) for triterpenes and benzene-acetic acid-water (125:72:3) for flavonoids, visualized

under I_2 vapors. Spots coinciding to reference compounds were marked, scrapped, eluted with chloroform and ethyl acetate respectively. Each of elutes was dried, reconstituted in chloroform and crystallized using methanol. Later, each of the isolated compounds was subjected to melting point in capillary tubes (Toshniwal melting point apparatus) and IR (KBr pellets on A 400S Shimadzu FTIR spectrometer) spectral studies.

Quantification of the isolates

The concentration of phytosterols in different ages of callus cultures were estimated spectrophotometrically using the method of Das and Benerjee (1980). After adding chromogenic reagent optical density was measured in spectrophotometer at 540 nm against a blank. Flavonoids were estimated using $AlCl_3$ spectrophotometric method (Mabry *et al.,* 1970). After adding chromogenic reagent the optical density was taken at 426 nm for kaempferol and 440 nm for quercetin. Respective contents in each case were calculated in terms of mg g^{-1} dw from the computed standard calibration curve.

Antioxidant efficacies

By 2, 2-Diphenyl-1-picryl-hydrazyl (DPPH) method

The effect on DPPH radical was determined using the method of Fogliano *et al.* (1999). After 30 minutes of incubation time, OD was measured at 517 nm. Quercetin was used as standard. The capability to scavenge the DPPH radical was calculated using following equation.

$$\% \text{ Inhibition} = 1-(OD_{Sample}/ OD_{Control}) \times 100$$

Where, $OD_{Control}$ is the absorbance of the control (containing all reagents except the test compounds) and OD_{Sample} is the absorbance of test sample. The OD of control was considered as 100% unreduced DPPH and IC_{50} values were determined as the concentration of the extract required to achieve 50% reduction in DPPH radicals.

By ferric ion reducing antioxidant potentials method

Total reducing power of extracts was determined according to FRAP method (Yen and Chan, 1995). After addition of chromogenic reagent absorbance was measured at 700 nm. A standard calibration curve of ascorbic acid (10-500 mg ml^{-1}) was prepared and antioxidant activity was expressed in mg of ascorbic acid equivalents (mg AAE g^{-1}) of extract.

Antimicrobial efficacy

For antimicrobial screening, pure cultures of test bacteria and fungi were obtained from IMTECH, Chandigarh, India. Antimicrobial assay was performed by agar well diffusion method (Boyanova *et al.,* 2005). 40 µl (4 mg) of the extracts were filled into each of the wells and incubated at 37±0.2°C. Antibacterial activity in terms of zones of inhibition (mm) was recorded after 24 hr of incubation. All experiments were performed in triplicate. Gentamycin (10 mcg $disc^{-1}$) for bacteria and ketonocozole (10 mcg $disc^{-1}$) for fungi were used as positive controls.

Statistical analysis

All experiments were performed in triplicates and data were analyzed according to the One-Way ANOVA method using SSPS 10.0 (USA) program. Mean values (three replicates ± SD) were statistically compared with Ducan test, at probability level $P \leq 0.05$.

RESULTS AND DISCUSSION

Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.,* 1999). Since long, tissue culture studies in medicinal plants have been conducted for the production of secondary metabolites (phenylpropanoids, alkaloids and terpenoid metabolites, etc.). Rapid rate of (plant) species extinction, cell, tissue and organ culture pave a way for alternative aseptic, controllable, automatized way for the production of secondary metabolites.

In present study, leaf explants produced a mass of friable callus on MS medium supplemented with 2,4-D (2 mg l^{-1}) and Kn (0.5 mg l^{-1}) within 30 days of incubation. Callus was profuse and white friable in nature. White callus turned yellow after 6 weeks and green after 8 weeks of sub-culturing. Purple-red colored cell clusters appeared spontaneously in one of the wild cell line cultures. This sector was selectively excised and subcultured on to the fresh medium. The frequency of pigmented cells gradually increased with each subculture cycle. Following this continuous cell aggregate selection technique, a more or less uniformly stained, dark purple pigmented callus line was established.

On the basis of TLC behavior, major spots (Rf 0.06, 0.20, 0.32, 0.36 and 0.26 in solvent system of benzene-heptane-alcohol, 100:100:1) were observed similar to the position as of markers used under UV light, to α-amyrin, β-amyrin, β-sitosterol, lupeol, lupeol acetate and oleanolic acid. Later, these spots gave similar color reactions with anisaldehyde reagent. On flavonoids analysis, two spots coincided to the reference kaempferol and quercetin (Rf 0.86 and 0.78 in solvent system of benzene-acetic acid-water, 125:72:3). These spots gave positive reactions to the spraying reagents used. The spots corresponding to standard markers were isolated by PTLC and dissolved in methanol. A comparison of TLC behavior, physical data and spectral properties was made and found to be accordance with those reported for the authentic samples (Table 1). On β-sitosterol estimation, higher levels were recorded in 6 weeks-old callus (83.45 ± 0.02 µg g^{-1} dw), whereas α- and β-amyrins were in 8 weeks-old callus (87.77 ± 0.56 and 82.85 ± 0.00 µg g^{-1} dw respectively). On flavonoids quantification, 4 weeks-old callus exhibited higher levels of kaempferol and quercetin (45.82 and 42.67 µg g^{-1} dw respectively). Similar to terpenoids, levels of flavonoids increased with the age attaining maximum at 4 weeks-old age followed by their decline (Table 2). Due to poor levels quantification of lupeol, lupeol acetate and oleanolic acid could not be made.

Antioxidant activity of different age groups, positively correlated to phenolics and flavonoids levels. Higher levels of phenolics and flavonoids were reported in 4 week callus (12.86 ± 0.61 mg GAE g^{-1} dw and 17.1 ± 0.10 mg QE g^{-1} dw respectively) with lower IC_{50} values 0.060 (% inhibition 90.93 ± 0.76 at 0.8 mg ml^{-1} concentration). Two weeks-old callus exhibited significant measure of phenolics (10.86 ± 2.13 mg GAE g^{-1} dw) and flavonoids (14.0 ± 0.08 mg QE g^{-1} dw) which are attributes to lower IC_{50} (0.065) and higher % inhibition 92.41 ± 1.57 at 0.08 mg ml^{-1} concentration (Table 3).

Similarly in FRAP method, 4 weeks-old callus extract exhibited higher levels of antioxidant potentials followed by 2 weeks-old callus extract (336.66 ± 2.14 and 255.00 ± 8.66 mg AAE g^{-1} dw). These extracts exhibited similar activity as the whole plant extract (263.33 ± 2.57 mg AAE g^{-1} dw). It was noteworthy that i*n vivo* plant almost similar levels of phenolics and flavonoids and antioxidant capacity like the 4 weeks-callus extracts were exhibited (Table 4).

Whole plant extract was found to be more active than callus extract (IZ 12.00 ± 0.57 and 13.00 ± 1.00 mm for *B. subtilis* and *E. aerogenes* respectively). On comparison among callus extracts, 6 weeks-old callus demonstrated maximum inhibition against *B. subtilis* (IZ 12.66 ± 0.57 mm), *E. aerogenes* (12.33 ± 0.57 mm) and *S. aureus* (12.00 ± 0.00 mm), while 10 weeks-old callus inhibited *S. aureus* with IZ of 12.00 ± 0.00 mm (Table 5). However, callus extract was poorly active against fungi. However, at 4 mg concentration, the callus extract did not inhibit any of the test fungi.

Terpenoids are the primary constituents of the essential oils of many types of plants and flowers and used widely as natural flavor food additives and in traditional medicines. Amyrins were found to exhibit interesting pharmacological activities such as anti-inflammatory, antiproteolytic, and antifungal (Chaturvedi *et al.*, 1974; Nes and Patterson, 1981). Stigmasterol has been reported to have antibacterial, antirheumatic (Gómez, 2001); β-sitosterol antimicrobial, antioxidant, anti-inflammatory, analgesic, antipyretic (Gupta *et al.*, 1980; Weng and Wang, 2000; Salvador *et al.*, 2004); lupeol, lupeol acetate cardioprotective, hepatoprotective, antimicrobial (Andrikopulos *et al.*, 2003; Flekhter *et al.*, 2000; Chaaib *et al.*, 2003), and oleanoic acid having antifungal and anti-inflammatory effects (Jeong *et al.*, 1999; Tang *et al.*, 2000). Flavonoids, quercetin and kaempferol are known for antioxidants, antiinflammatory, antiallergic, antiviral, antimicrobial as well as anticancer activities (Hillwell, 1994; Arima *et al.*, 2002; Lin *et al.*, 2005; Sung *et al.*, 2006).

Table 1: Chromatographic and spectroscopic data of the isolated compounds from *P. daemia in vivo* and *in vitro*

Isolated compounds	Rf in solvent systems*						m.p. (ºC)	IR (ν_{amx}) cm-1(KBr)
	I	II	III	IV	V	VI		
α-Amyrin	-	-	0.26	-	-	-	183-184	3400, 2990, 2850, 1640, 1480, 1380, 1360, 1200, 1120, 1050, 1030, 990, 960, 930, 890, 820
β-Amyrin	0.98	0.97	0.20	-	-	-	196-198	3350, 1650, 1190, 1140, 1100, 1050
Lupeol	-	-	0.36	-	-	-	-	3311, 2946, 2870, 1638, 1464, 1189, 1035, 996, 680
Lupeol acetate	-	-	0.32	-	-	-	-	2942, 2866, 1735, 1451, 1379, 1243, 1027, 996, 680
Oleanolic acid	-	-	-	0.75	-	-	297-298	3432, 2948, 1695, 1463, 1387, 1182, 1029
β-Sitosterol	0.95	0.96	0.06	-	-	-	139-141	3400, 2900, 2830, 1640, 1470, 1370, 1300, 1090, 1050, 1020, 820
Kaempferol	-	-	-	-	0.86	0.83	275-276	3320, 1660, 1610, 1570, 1500, 1440, 1390, 1300, 1250, 1220, 1180, 1090, 1010, 980, 880, 850, 820
Quercetin	-	-	-	-	0.78	0.64	275-276	3280, 1740, 1670, 1610, 1520, 1430, 1360, 1210, 1090, 1000, 930, 880, 810, 700, 590

**Solvent system: I- Hexane-acetone (4:1); II- Benzene-acetone (2:1); III- Benzene-heptane-alcohol (100:100:1); IV - Hexane-ethyl acetate (4:1); V- Benzene-acetic acid-water (125:72:3); VI- n-Butanol-acetic acid-water (4:1:5; upper layer).*

Table 2: **Quantification of isolated compounds in callus and *in vivo* plant (concentration of isolated compounds in µg g^{-1} dw**

Isolated compounds	Age of callus in weeks					*In vivo* plant
	2	4	6	8	10	
α-Amyrin	22.36 ± 0.05	31.58 ± 0.28	59.54 ± 0.59	87.77 ± 0.56	33.11 ± 0.67	32.11 ± 0.29
β-Amyrin	50.33 ± 0.00	65.44 ± 0.00	64.00 ± 0.00	82.85 ± 0.00	80.45 ± 0.00	58.33 ± 0.00
β-Sitosterol	56.33 ± 0.01	59.13 ± 0.01	83.45 ± 0.02	52.32 ± 0.02	50.69 ± 0.00	76.23 ± 0.01
Kaempferol	36.96 ± 0.37	45.82 ± 0.00	35.66 ± 0.17	38.22 ± 0.11	35.61 ± 0.58	45.36 ± 0.11
Quercetin	35.88 ± 0.11	42.67 ± 0.00	24.11 ± 0.15	28.41 ± 0.58	28.76 ± 0.58	42.33 ± 0.66

Data were statistically analyzed and representing mean ± standard deviation of three replicates per experiment(s).

Table 3: *In vitro* antioxidant activity of callus and whole plant extracts of *P. daemia* by DPPH method

Nature of alcoholic extract	Total phenolics (mg GAE g^{-1} dw)[a]	Total flavonoids (mg QE g^{-1} dw)[b]	IC_{50}	[c]% Inhibition (concentration in mg ml^{-1})				
				0.1	0.2	0.4	0.6	0.8
2 wks-old callus	10.86 ± 2.13	14.0 ± 0.08	0.065	84.55 ± 0.90	87.70 ± 0.85	89.82 ± 1.35	93.94 ± 0.25	92.41 ± 1.57
4 "	12.86 ± 0.61	17.1 ± 0.10	0.060	78.32 ± 1.12	79.58 ± 1.65	85.34 ± 0.28	87.40 ± 0.75	90.93 ± 0.76
6 "	10.60 ±1.03	9.50 ± 0.05	0.080	66.39 ± 2.30	66.43 ± 0.44	67.19 ± 0.92	72.10 ± 1.10	73.33 ± 0.79
8 "	10.26 ± 1.21	23.8 ± 0.05	0.100	64.05 ± 4.05	66.77 ± 3.37	74.19 ± 2.44	77.19 ± 0.34	78.96 ± 0.65
10 "	10.08 ± 0.41	13.1 ± 0.05	2.750	42.47 ± 0.59	46.57 ± 6.67	48.64 ± 3.09	55.33 ± 1.92	58.07 ± 1.18
In vivo plant	13.26 ± 0.46	23.1 ± 0.05	0.080	84.55 ± 0.90	87.70 ± 0.85	89.82 ± 1.35	93.94 ± 0.25	92.41 ±1.57
Quercetin	-	-	0.040	62.42	80.58	93.38	93.82	94.71

[a]*Total phenolics content in mg gallic acid equivalent g^{-1} dry weight (GAE g^{-1} dw);* [b]*Total flavonoids content in mg quercetin equivalent/g dry weight (mg QE g^{-1} dw);* [c]*% Inhibition = 1-(Absorbance of the sample/Absorbance of the control) × 100.*

Table 4: *In vitro* antioxidant activity of callus and whole plant of *P. daemia* by FRAP method

Nature of alcoholic extract	Antioxidant activity in mg AAE g^{-1} dw[a] (concentration in µg ml^{-1})				
	62.5	125	250	500	1000
2 wks-old callus	28.66 ± 5.50	31.66 ± 2.88	46.66 ± 2.88	70.66 ± 6.92	255.00 ± 8.66
4 "	25.00 ± 0.00	28.66 ± 5.50	31.66 ± 2.88	50.00 ± 5.00	336.66 ± 2.14
6 "	24.66 ± 0.57	25.00 ± 0.00	28.33 ± 2.88	43.33 ± 2.88	281.66 ± 5.77
8 "	25.00 ± 0.57	30.00 ± 0.00	43.33 ± 2.88	78.66 ± 5.50	203.66 ± 16.07
10 "	25.66 ± 0.57	30.00 ± 0.00	45.00 ± 0.00	55.00 ± 8.66	206.66 ± 5.77
In vivo plant	35.00 ± 5.00	46.66 ± 2.88	55.00 ± 8.66	121.66 ± 25.16	263.33 ± 2.57
Ascorbic acid	62.5	125	250	500	1000

[a] *mg AAE g^{-1} = mg Ascorbic acid equivalent g^{-1} extract.*

Table 5: Antimicrobial activity of callus and whole plant ethanolic extract of *P. daemia*

Test microorganisms	MTCC No.		Callus extract (age in weeks)					Whole plant extract
			2	4	6	8	10	
B. subtilis	441	IZ[a]	10.66 ± 0.57	11.66 ± 1.66	12.66 ± 0.57	11.66 ± 0.57	10.33 ± 0.57	12.00 ± 0.57
		AI[b]	0.48	0.53	0.57	0.53	0.46	0.54
E. aerogenes	111	IZ	10.00 ± 0.00	10.66 ± 0.57	12.33 ±0.57	10.00 ± 0.00	10.00 ± 0.00	13.00 ± 1.00
		AI	0.71	0.76	0.88	0.71	0.71	0.92
E. coli	443	IZ	8.00 ± 0.00	8.00 ± 0.00	9.00 ±0.00	8.66 ± 0.57	11.33 ± 0.57	7.00 ± 0.00
		AI	0.42	0.42	0.47	0.45	0.59	0.36
K. pneumoniae	109	IZ	-	-	-	-	-	-
		AI	-	-	-	-	-	-
R. planticola	530	IZ	-	8.33 ± 0.57	11.33 ± 0.57	10.33 ± 0.57	10.33 ± 0.57	11.33 ± 1.15
		AI	-	0.37	0.51	0.46	0.46	0.51
S. aureus	740	IZ	8.00 ± 0.00	11.33 ± 1.15	12.00 ± 0.57	10.66 ± 1.15	12.00 ± 0.00	8.00 ± 0.00
		AI	0.38	0.53	0.57	0.50	0.57	0.38
C. albicans	16870	IZ	-	-	-	-	-	-
		AI	-	-	-	-	-	-
P. chrysogenum	5476	IZ	-	-	-	-	-	-
		AI	-	-	-	-	-	-
T. rubrum	2327	IZ	-	-	-	-	-	-
		AI	-	-	-	-	-	-

Test samples 4 mg well^{-1}; Standard test drugs: Gentamycin for bacteria, Ketonocozole for fungi (10 mcg disc^{-1}); [a]IZ=Inhibition zone (in mm) including the diameter of well (6 mm); [b]AI= Activity index = Inhibition zone of the sample/Inhibition zone of the standard.

CONCLUSION

In the present studies, the plant cell cultures demonstrated broad-spectrum antibacterial and antioxidant efficacies. Cell cultures reported appreciable levels of triterpens as well as flavonoids compounds. Total phenolics and flavonoids levels in callus extract were lower than whole plant extract. However, 4 weeks-old callus extract exhibited antimicrobial and antioxidant potentials significantly similar to whole plant extract. As the plant has been used for many years in the preparation of decoction/infusions to treat various ailments, its cell culture due to the presence of similar types of compounds, provides a scientific basis for its use as an alternate.

REFERENCES

Andrikopoulos NK, Kaliora AC, Assimopoulou AN & Papapeorgiou VP (2003). Biological activity of some naturally occurring resins, gums and pigments against *in vitro* LDL oxidation. *Phytotherapy Research,* **17** 501-507.

Anjaneyulu ASN, Raju DVSN, & Srinivasa Rao S (1998). Chemical evaluation of *Pergularia daemia*. *Indian Journal of Chemistry*, **37B** 318-320.

Arima H, Ashida H, & Danno G (2002). Rutin-enhanced antibacterial activities of flavonoids against *Bacillus cereus* and *Salmonella enteritidis*. *Bioscience, Biotechnology and Biochemistry*, **66** 1009-1014.

Baydar NG, Öçzkan G & Sagdiç O (2004). Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts. *Food Control* **15** 335–339.

Bernays EA, Cooper Driver G, & Bilgener M (1989). Herbivores and plant tannins. *International Advances in Economic Research.* **19** 263–302.
Boyanova L, Gergova G, Nikolov R, Derejian S, Lazarova E, Katsarov N, Mitov, I., & Krastev Z. (2005). Activity of Bulgarian propalis against 94 *Helicobacter pylori* strains *in vitro* by agar well diffusion, agar dilution and disc diffusion methods. *Journal of Medical Microbiology,* **54(5)** 481-483.
Bray HG, & Thorpe WV, (1954). Methods of Biochemical Analysis. In: Glick, D. (eds.). John Wiley & Sons, New York, USA. pp 27-57.
Chaaib F, Queiroz EF, Ndjoko K, Diallo D & Hostettmann K (2003). Antifungal and antioxidant compounds from the root bark of *Fagara zanthoxyloides. Planta Medica,* **69** 316-320.
Chaturvedi A, Chaturvedi AK, Parmar SS, & Brumleve SJ (1974). Antiproteolytic activity of amyrin acetate. *Research Communications Chemical Pathology and Pharmacology,* **7** 205-208.
Chung KT, Wong TY, Wei CI, Huang YW, & Lin Y (1998). Tannins and human. health: a review. *Critical Review in Food Science and Nutrition* **38** 421.
Cushnie T, & Lamb A (2005) Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents,* **26** 343–356.
Das SK, & Banerjee AB (1980). A rapid method for quantification of sterols after thin layer chromatography. *Indian Journal of Experimental Biology,* **18** 969-971.
Flekhter OB, Karachurina LT, Poroikov VV, Nigmatullina LP, Baltina LA, Zarudii FS, Davydova VA, Spirikhin LV, Balikova IP, Galin FZ, & Tolstikov GA (2000). The synthesis and hepatoprotective activity of esters of lupine group triterpenoids. *Russian Journal of Bioorganic Chemistry,* **26** 192-200.
Fogliano V, Verde V, Randazzo G & Ritieni A (1999). A method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. *Journal of Agriculture and Food Chemistry,* **47** 1035-1040.
Golam Sadik Md, Gafur MA, Shah Alam Bhuiyan M, Rahman MM, & Biswas MHU (2001). Antifertility Activity of the alkaloidal fraction of *Pergularia daemia*. *Journal of Medical Science,* **1(4)** 217-219.
Gómez MA, García MD, & Sáenz MT (2001). Cytostatic activity of *Achillea ageratum* L. *Phytotherapy Research,* **15(7)** 633–634.
Gupta AK, Vidyapati TJ, & Chauhan JS (1980) Chemical examination of stem of *Bauhinia variegata*. *Planta Medica,* 38 174-176.
Gupta MB, Bhalla TN, Tangri KK, & Bhargava KP (1971). Biochemical study of the anti-inflammatory activity of α- and β-amyrin acetate. *Biochemistry and Pharmacology,* **20** 401-405.
Hillwell B (1994). Free radicals, antioxidants and human disease: Curiosity, cause or constipation? *Lancet,* **344** 721-724.
Jain SC, Sharma RA, Jain R, Mascolo N, Capasso F, Vijayvergia R, & Mittal C (1998). Ethnopharmacological evaluation of *Pergularia daemia* (Forsk) Chiov. *Phytotherapy Research,* **12(3)** 378-380.
Jeong TS, Hwang EI, Lee HB, Lee ES, Kim YK, Min BS, Bae KH, Bok SH, & Kim SU (1999). Chitin synthase II inhibitory activity of ursolic acid, isolated from *Crataegus pinnatifida*. *Planta Medica,* **65** 261-263.
Kerharo J & Adam JG (1974). La pharmocopée sénégalaise tradionnelle. Plantes médicinals et toxique. Vigot Frères, Paris, France. pp 1011.
Kerharo J & Boquet A (1950). Plants médicinales et toxiques de la Côte-d'Ivoir – Haute-Volta. In: Mission d'étude de la pharmacopée indigène en A.O.F. (eds.) Vigot Frères, Paris. 300.
Kirtikar KR & Basu BD (1983). Indian Medicinal Plant. Part II. International Book Distributors, Dehradun, India. pp 1616-1617.
Lin RD, Chin YP & Lee MH (2005). Antimicrobial activity of antibiotics in combination with natural flavonoids against clinical extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae*. *Phytotherapy Research,* **19** 612-617.

Mabry TJ, Markham KR & Thomas MG (1970). The Systematic Identification of Flavonoids. Springer Verlag, Berlin.
Miyake Y, Murakami A, Sugiyama Y, Isobe M, Koshimizu K, & Ohigashi K (1999). Identification of coumarins from lemon fruit citrus limon as inhibitors of *in vitro* tumor promotion and superoxide and nitric oxide generation. *Journal of Agriculture and Food Chemistry,* **47** 3151–3157.
Murashige T, & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15** 473-497.
Nayar SL, Chopra RN & Chopra IC (1956). Glossary of Indian Medicinal Plants, CSIR, New Delhi, India. pp 188.
Nes WD, & Patterson GW (1981). Effects of tetracyclic and pentacyclic triterpenoids on growth of *Phytophthora cactorum*. *Journal of Natural Product* **44** 215-220.
Salvador MJ, Zucchi OLAD, Candido RC, Ito IY, & Dias DA (2004). *In vitro* antimicrobial activity of crude extracts and isolated constituents of *Alternanthera maritime* (Amaranthaceae). *Pharmaceutical Biololgy,* **42** 499-505.
Santhilkumar M, Gurumoorthi P, & Janardhanan K (2004). Antibacterial potential of some plants used by the tribals in Maruthamalai hills, Tamil Nadu. *National Institute of Sciences Communication and Information Resources,* **4(1)** 27-34.
Schanderl SH (1970). Tannins and related phenolics. In: Joslyn M.A. (ed.): Methods in Food Analysis. Academic Press, New York. pp 701–724.
Sung WS, Jung HJ, Lee IS, Kim HS & Lee DG (2006). Antimicrobial effect of furaneol against human pathogenic bacteria and fungi. *Journal of Microbiology and Biotechnology,* **16** 349-354.
Sureshkumar SV & Mishra SH (2006). Hepatoprotective activity of extract from *Pregularia daemia. Journal of Ethnopharmacology* **107(2)** 164-168.
Tang HQ, Hu J, Yang L, & Tan RX (2000). Terpenoids and flavonoids from *Artemisia* sp. *Planta Medica,* **66** 391-393.
Wahi AK, Ravi J, Hemalatha S & Singh PN (2002). Antidiabetic activity of *Daemia extensa. Journal of Natural Remedies,* **2(1)** 80-83.
Wang SY, & Jiao H (2000). Scavenging Capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *Journal of Agriculture and Food Chemistry,* **48** 5677–5684.
Weng XC & Wang W (2000). Antioxidant activity of compounds isolated from *Salvia plebeia*. *Food Chemistry,* **71** 489–493.
Williams RL, & Elliot MS (1997). Antioxidants in grapes and wine: chemistry and health effects. In: Shaihidi F (ed.): Natural Antioxidants: Chemistry, Health Effects and Applications. AOCS Press, Illinois. pp 150–173.
Yen GC, & Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. *Journal of Agriculture and Food Chemistry,* **43** 27-32.
Zhishen J, Mengcheng T & Jianming W (1999). The determination of flavonoid content in mulberry and their scavenging effect on superoxide radicals. *Food Chemistry,* **64(4)** 555-559.