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HPTLC FINGER PRINT ANALYSIS OF PHYTOPHENOLS OF *PAEDERIA FOETIDA* UNDER DIFFERENT EXTRACTION REGIMEN

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ABSTRACT
To detect and estimate the phenolics and flavonoids of *Paederia foetida* in different solvents under CP, MAE system and by HPTLC finger print analysis. Phenolic components in chlorophyll free extracts in different solvent groups were qualitatively detected by chemical tests and total polyphenols and flavonoids were quantitatively estimated following standard protocol. Distinct chemo-profile of the components was analyzed by HPTLC technique with CAMAG *win CATS* planar chromatography manager software. Among two methods of extraction more components of polyphenols were detected and significantly higher ($p<0.05$) contents of phenolics and flavonoids were estimated in MAE method than CP method. Out of five solvent types, organic solvent mixtures recovered significantly higher polyphenols ($p<0.05$) and flavonoids ($p<0.001$) in comparison to aqueous solvent. CP Method exhibits 12, 14, 14, 14 and 16 peaks for Gr-A, B, C, D and E solvents respectively. In MAE method the numbers of peaks in these respective solvent groups are 15, 21, 19, 18 and 22. Phyto-chemicals can be better screened qualitatively and estimated quantitatively in organic solvent extracts under MAE system by HPTLC finger print analysis than the aqueous solvent and CP method.
Abbreviations: CP: Cold Percolation, MAE: Microwave-Assisted Extraction, R_f: Retention factor, HPTLC: High Pressure Thin layer Liquid Chromatography.

INTRODUCTION

The scientific world, in recent era of medicine emphasizes on phyto-constituents as lead molecules in development of future generation safe drugs. This is because, large diversity of phytophnols and flavonoids lack adverse effects and possess free radical scavenging activity to reduce risks of cancer, cardiovascular disease, diabetes, inflammation, bacterial disease and ageing related diseases (Kumar and Surh, 2008). Phyto-constituents vary in chemical characteristics, polarities and distribution in the plant matrix (Sultana *et al.,* 2009) and their recovery depends on extraction techniques and the nature/ volume of solvent (Turkmen *et al.*, 2006). Conventional extraction techniques with water as solvent recover less constituent and is time consuming. On the other hand, advanced technique of microwave assisted extraction with polar solvents consumes less time and solvent and claims to extract even thermo-labile constituents also (Mandal *et al.*, 2007). The qualitative and quantitative analysis of phyto-constituents by different methods exhibits the efficacy of the technique and the solvent of extraction (Behera *et al.,* 2012b). High Pressure Thin Layer Chromatography (HPTLC) finger print is a step ahead of such analysis with constitutional details. So, screening of these techniques and solvents on recovery of different phyto-constituents by HPTLC finger print is more suitable than the traditional qualitative and quantitative estimation (Behera *et al.,* 2012a).

Water being non-polar solvent fails to extract all active principles and thereby potency of the phyto-constituent suffers to be suppressed. Sometimes, conventional extraction method with water is equally responsible for low potency of herbal formulations. The plants may have some more components to be extracted for better performance. So, there is a need to compare the constituents recovered in different methods and solvents for potency augmentation of phyto-medicines.

Paederia foetida (shunk vine) of Rubiaceae family is an aromatic perennial climbing shrub. It is native to eastern Asia and grown in grassy hillsides, forests, river banks, waste grounds, roadsides on fences and urban areas of Assam, Bihar, Bengal and Odisha. It is a well known medicinal shrub for its antibacterial (Senapati *et al*., 2013a), Cytotoxic (Morshed *et al*., 2012), anthelmintic (Dey and Pal, 2011), anti-hyperglycemic (Khan *et al*., 2011), hepato-protective (Uddin *et al.,* 2011), anti-fungal (Majumdar *et al*., 2011), anti-ulcer (Reddy *et al*., 2011), antioxidative (Osman *et al*., 2009) and anti-diarrhoeal (Afroz *et al*., 2006) activities in several countries. Indian tribes of Orissa province use aqueous paste of this shrub traditionally for treatment of rheumatoid arthritis, hepatic disorders, piles, diabetes, asthma, coughs, body ache, itches, wounds, stomach-ache, diarrhoea, dysentery, flatulency and toothache.

Therefore, the present study aims to screen the phenolic components of *Paederia foetida* shoot extracted in different groups of solvents following the methods of Cold Percolation (CP) and Microwave Assisted Extraction (MAE) through qualitative, quantitative and HPTLC finger print analysis with an objective to advocate the suitable technique and solvent for better extraction of phyto-constituents.

MATERIALS AND METHODS

Plant sample

Paederia foetida collected from different regions of Khurdha districts of Odisha Province was identified and classified following the description of Saxena and Brahman (1995). The whole shoot of the plant was collected at pre-flowering stage, cleaned, dried under shade and ground into fine structure for preparation of extracts.

Solvents

Organic and aqueous solvents and their mixtures were classified under Gr-A: Distilled and deionised water @100 %, Gr-B: Methanol: Water, Gr-C: Ethanol: Water and Gr-D: Acetone: Water @ 80:20 % each and Gr-E: Methanol: ethanol: acetone: water @ 30:30:30:10 %.

Extraction

Extraction of polyphenols and flavonoids was conducted in two trials. In trial-I, extraction of phyto-constituents was done in MAE system by Multiwave 3000-801V (Anton Paar) digestion system following the method of Eskilsson and Bjorklund (2000). 2g of ground powder in 20mL of solvent was heated at 80ºC for 25 minutes followed by 15 minutes cooling. In trial-II, the extraction of was done by cold percolation following the method as described by Senapati *et al*. (2013b). 2g of ground sample in 40mL of solvent was kept on magnetic stirrer at 10ºC temperature for 24h followed by filtration of the extract.

Hexane treatment

Equal volumes of filtered crude extract and hexane were mixed and kept for 2 minutes. The supernatant was aspirated carefully to obtain chlorophyll free extract.

Qualitative detection of phyto-constituents

The phenolic components in shoot extracts were detected by specific chemical tests as detailed below.

(a)Salkowski's Test for Terpenoids: 1mL of extract + 2mL of chloroform + 1 mL of concentrated H_2SO_4. Reddish brown colour at the interface indicates positive result.

(b)Ferric Chloride's Test for Tannins: 1 mL extract + 1 mL of 0.1% $FeCl_3$. Brown-green/ blue black colour indicates positive result.

(c)Shinoda's Test for Flavonoids: 2 mL extract + few drops of 1% NH_3 solution. Yellow colour indicates positive result.

(d)Killer-killani's Test for Cardiac glycosides: 5 mL of extract + 2 mL of glacial CH_3COOH containing 1 drop of 0.1% $FeCl_3$ + 1 mL of concentrated H_2SO_4. Brown colour ring at the interface indicates positive result.

(e)Frothing Test for Saponins: 10 mL of extract + 5 mL of distilled water. The shaked froth was mixed with 3 drops of olive oil. Formation of emulsion indicates positive result.

(f) Liebermann-Burchard's Test for Steroids: 1 mL of extract + 2 mL of acetic anhydride + 2 mL of concentrated H_2SO_4. Colour change from violet to green indicates positive result.

(g)Wagner's Test for Alkaloids: 5 mL of extract + 2 mL of Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/ reddish precipitate indicates positive result.

Qunantitative estimation of Phenolics and flavonoids

The shoot extracts in different solvents were estimated for total polyphenols (Singh *et al.,* 2002) and flavonoids (Mimica-Dukic, 1992) by chemical exposure followed by spectro-photometric assessment of optical absorbance.

HPTLC Finger printing of phenolic components

Distinct chemo-profile of the extract was studied by HPTLC technique following the method of Stahl (2007). The silica gel TLC plates (EMerck, Germany) of $60F_{254}$ (20x10x 0.0002cm) were spotted with 5 μL of extracts in the form of band with HPTLC glass syringe and dried at 60-70ºC by CAMAG TLC plate Heater-III for 60-90 seconds. The CAMAG Automatic Developing Chamber-2 (ADC-2) was saturated with the 50 mL. of mobile phase (Toluene: Ethyl acetate: Formic acid (8.5: 1.0: 0.5) for 10 min at room temperature (25 $\pm$ 2ºC). The plates were exposed to mobile phase in the ADC-2 till movement of solvent occurs up to 95% of the plate. Then the plates, after air drying for 10 minutes, were scanned by CAMAG-TLC scanner-3 in remission-absorbance mode at 254nm using optical filter K400, under control of Camag *win CATS* planar chromatography manager software with specific slit dimension of 5$\times$ 0.45mm, the sample track and spot spectrum scanning speed @ 20 mm/ sec, band length of 5mm and tracks distance of 8.9mm.

Statistical analysis

The data was subjected to analysis of variance to test the significance of difference of mean values between different groups according to Snedecor and Cochran (1994) and by using *Instat*-3 software.

RESULTS

Detection of phenolic compounds

The qualitative detection of phenolic compounds in chlorophyll free shoot extracts of *Paederia foetida* in five different solvents (Table 1) reveals that flavonoid, terpenoid, steroid and alkaloid are absent in CP method where as all the constituents under study present in MAE method except terpenoid and alkaloid at Gr-A. Organic solvent mixtures at Gr-B, C, D and E extract all the components in MAE method but CP method excludes terpenoids, saponins and alkaloids in the extract. MAE method and solvent mixture at Gr-E depicts presence of more phyto-constituents as compared to their counter groups.

Estimation of phenolics and flavonoids by different methods

Table 2 compares the contents of total phenolics and flavonoids extracted in five different solvents under MAE and CP methods. MAE method extracts significantly higher contents ($p < 0.05$) of total phenolics in all the solvents and the maximum concentration (3.98 $\pm$ 0.31) is recovered in Gr-E solvent followed by Gr-B (3.72 $\pm$ 0.29), C (3.58 $\pm$ 0.21), D (3.42 $\pm$ 0.22) and A (2.93 $\pm$ 0.18) than those extracted under CP method. In addition, flavonoids extracted in MAE method depicts significantly higher contents ($p < 0.05$) in all the groups but solvent mixture in Gr-E extracts the maximum concentration (1.05 $\pm$ 0.10) followed by Gr-C (0.93 $\pm$ 0.06), B (0.78 $\pm$ 0.07), D (0.69 $\pm$ 0.08) and A (0.49 $\pm$ 0.05) in comparison to the corresponding contents observed in CP method. MAE method extracts significantly higher contents of

Table 1: Detection of phenolic constituents of *Paederia foetida* shoot extracted in different solvents

Methods	CP	MAE	CP	MAE	CP	MAE	CP	MAE	CP	MAE
Solvent groups	Gr-A		Gr-B		Gr-C		Gr-D		Gr-E	
Tannin	+	+	+	+ +	+	+	+	+	+	+
Flavonoid	-	+	+	+ +	+	+	+	+	+	+ +
Terpenoid	-	-	-	+	-	+ +		+	+	+ +
Cardiac glycosides	+	+	+	+	-	+	+	+	+	+
Saponins	+	+ +	-	+	-	+	-	-	-	+
Steroids	-	+	+	+ +	+	+ +	-	+	-	+
Alkaloids	-	-	-	+	-	+	-	+	-	+ +

"+"and "–" denote presence and absence of the concerned components respectively.

Table 2: Total phenolics (mg of GAE / g of sample ± SE) and flavonoids (mg of RE / g of sample ± SE) in chlorophyll free shoot extracts of *Paederia foetida* in different methods

Solvents	Total polyphenols (mg of GAE / g of sample ± SE)		Total Flavonoid (mg of RE / g of sample ± SE)	
	MAE	CP	MAE	CP
Gr-A	$2.93^{a} \pm 0.18$	$2.07^{b} \pm 0.14$	$0.49^{a} \pm 0.05$	$0.27^{b} \pm 0.04$
Gr-B	$3.72^{a} \pm 0.29$	$2.22^{b} \pm 0.20$	$0.78^{a} \pm 0.07$	$0.46^{b} \pm 0.05$
Gr-C	$3.58^{a} \pm 0.21$	$2.76^{b} \pm 0.16$	$0.93^{a} \pm 0.06$	$0.52^{b} \pm 0.07$
Gr-D	$3.42^{a} \pm 0.22$	$2.47^{b} \pm 0.13$	$0.69^{a} \pm 0.08$	$0.36^{b} \pm 0.06$
Gr-E	$3.98^{a} \pm 0.31$	$3.10^{b} \pm 0.21$	$1.05^{a} \pm 0.10$	$0.71^{b} \pm 0.08$

Different superscripts between columns shows significant difference ($p<0.05$) within a group

Table 3: Total phenolics (mg of GAE / g of sample ± SE) and flavonoids (mg of RE / g of sample ± SE) in chlorophyll free shoot extracts of *Paederia foetida* in different solvents

		Gr-A	Gr-B	Gr-C	Gr-D	Gr-E
MAE	Total polyphenols	2.93 ± 0.18	3.72 ± 0.29	3.58 ± 0.21	3.42 ± 0.22	$3.98^{*} \pm 0.31$
	Total Flavonoid	0.49 ± 0.05	0.78 ± 0.07	$0.93^{**} \pm 0.06$	0.69 ± 0.08	$1.05^{***} \pm 0.10$
CP	Total polyphenols	2.07 ± 0.14	2.22 ± 0.20	2.76 ± 0.16	2.47 ± 0.13	$3.10^{**} \pm 0.21$
	Total Flavonoid	0.27 ± 0.04	0.46 ± 0.05	0.52 ± 0.07	0.36 ± 0.06	$0.71^{**} \pm 0.08$

Different superscripts between columns shows significant difference ($^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$) within a row.

Table 4: R_f values (Start and End Position) of peaks in different solvent extracts of *Paederia foetida* under CP method of extraction

Peaks	Gr-A		Gr-B		Gr-C		Gr-D		Gr-E	
	Start	End	Start	End	Start	End	Start	End	Start	End
1	0.02	0.09	0.01	0.04	0.01	0.03	0.01	0.03	0.01	0.03
2	0.09	0.12	0.04	0.07	0.03	0.07	0.03	0.04	0.03	0.07
3	0.14	0.16	0.10	0.13	0.09	0.12	0.04	0.07	0.07	0.09
4	0.17	0.18	0.14	0.17	0.12	0.16	0.07	0.08	0.18	0.23
5	0.18	0.22	0.18	0.22	0.19	0.22	0.15	0.16	0.23	0.24
6	0.25	0.28	0.26	0.28	0.25	0.28	0.26	0.27	0.29	0.33
7	0.44	0.47	0.33	0.34	0.34	0.36	0.40	0.43	0.38	0.40
8	0.53	0.56	0.35	0.39	0.43	0.47	0.60	0.62	0.40	0.41
9	0.57	0.61	0.44	0.47	0.51	0.53	0.64	0.68	0.46	0.49
10	0.64	0.68	0.47	0.53	0.57	0.59	0.70	0.72	0.51	0.54
11	0.85	0.87	0.66	0.69	0.61	0.63	0.72	0.77	0.55	0.57
12	0.88	0.92	0.73	0.78	0.69	0.71	0.78	0.81	0.57	0.62
13	-	-	0.84	0.86	0.73	0.78	0.84	0.88	0.65	0.69
14	-	-	0.88	0.93	0.83	0.94	0.89	0.95	0.71	0.74
15	-	-	-	-	-	-	-	-	0.76	0.82
16	-	-	-	-	-	-	-	-	0.83	0.96

phenolics and flavonoids than the traditional CP method in all the solvent groups.

Quantitative estimation of phenolics and flavonoids in different solvents

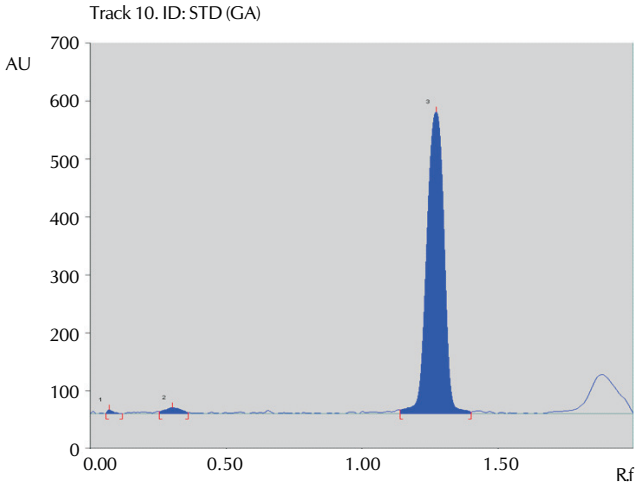
Higher content of total phenolics in Gr-E organic solvent extracts is significant in MAE ($p<0.05$) and CP method ($p<0.01$) in comparison to Gr-A aqueous solvent. The contents in rest of solvent groups are non-significant even though they exhibit more concentration in both the methods. The total flavonoids in MAE method is observed significantly higher at Gr-C ($p<0.01$) and Gr-E ($p<0.001$) but the value in CP method is found significant ($p<0.01$) only for Gr-E solvent (Table 3). Group E solvent mixture extracts significantly higher contents of total phenolics and flavonoids in both CP and MAE methods of extraction.

HPTLC analysis of polyphenols and flavonoids

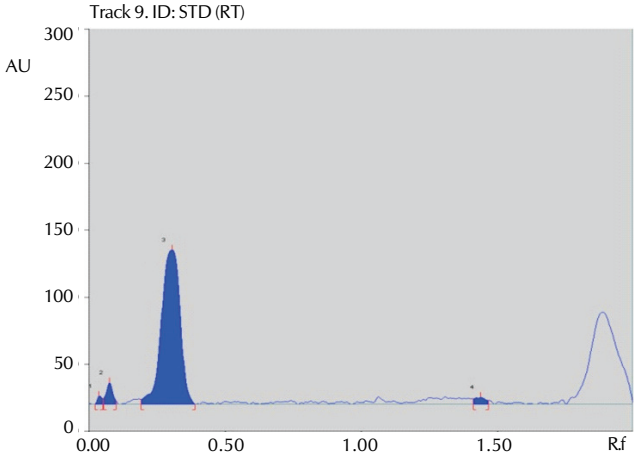
The chromatogram at Fig. 1a and 1b presents the spots of Gallic Acid (R_f 1.19-1.35) and Rutin (R_f 0.23-0.35) standards for polyphenols and flavonoids respectively. The shoot extracts of *Paederia foetida* under HPTLC depict variable number of peaks under MAE and CP method in different solvents. CP Method exhibits 12 (R_f 0.02- 0.92), 14 (R_f 0.01- 0.93), 14 (R_f

Table 5: R_f values (Start and End Position) of peaks in different solvent extracts of *Paederia foetida* under MAE method of extraction

Peaks	Gr-A		Gr-B		Gr-C		Gr-D		Gr-E	
	Start	End	Start	End	Start	End	Start	End	Start	End
1	0.01	0.04	0.01	0.03	0.01	0.03	0.01	0.03	0.01	0.03
2	0.04	0.08	0.03	0.04	0.03	0.09	0.03	0.08	0.04	0.09
3	0.11	0.14	0.04	0.06	0.09	0.12	0.12	0.14	0.14	0.15
4	0.17	0.21	0.19	0.21	0.15	0.17	0.16	0.18	0.17	0.19
5	0.22	0.24	0.23	0.25	0.27	0.29	0.20	0.23	0.20	0.22
6	0.25	0.28	0.25	0.27	0.29	0.32	0.26	0.31	0.26	0.29
7	0.30	0.34	0.30	0.32	0.34	0.35	0.34	0.35	0.29	0.32
8	0.42	0.46	0.32	0.37	0.36	0.41	0.40	0.42	0.33	0.35
9	0.49	0.51	0.38	0.41	0.42	0.47	0.44	0.46	0.35	0.38
10	0.61	0.66	0.42	0.44	0.47	0.50	0.46	0.49	0.39	0.41
11	0.69	0.70	0.47	0.49	0.51	0.56	0.51	0.53	0.42	0.46
12	0.73	0.75	0.50	0.52	0.58	0.60	0.57	0.61	0.46	0.50
13	0.83	0.86	0.53	0.56	0.62	0.67	0.67	0.70	0.50	0.53
14	0.88	0.89	0.57	0.60	0.69	0.71	0.72	0.78	0.58	0.60
15	0.91	0.95	0.61	0.64	0.72	0.79	0.78	0.81	0.60	0.63
16	-	-	0.64	0.65	0.81	0.84	0.81	0.84	0.66	0.69
17	-	-	0.65	0.66	0.84	0.87	0.85	0.87	0.72	0.75
18	-	-	0.69	0.71	0.88	0.90	0.89	0.94	0.77	0.79
19	-	-	0.74	0.78	0.90	0.94	-	-	0.79	0.82
20	-	-	0.79	0.82	-	-	-	-	0.84	0.86
21	-	-	0.82	0.86	-	-	-	-	0.87	0.88
22	-	-	-	-	-	-	-	-	0.89	0.94

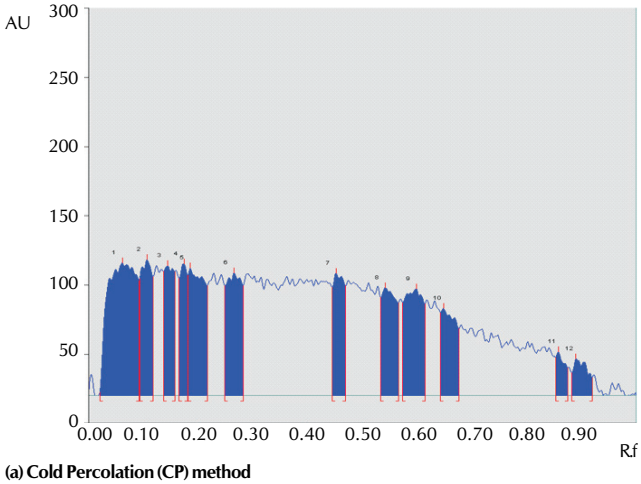


(a) Gallic Acid Standard 100mcg/ mL

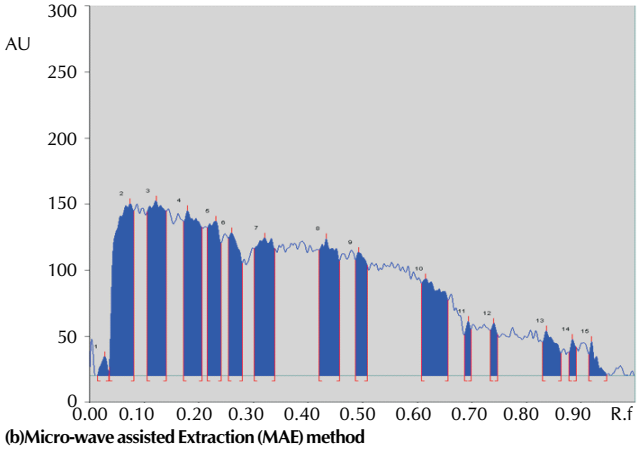


(b) Routin Standard 100mcg/ mL

Figure 1: HPTLC chromatogram of gallic acid and routin standand

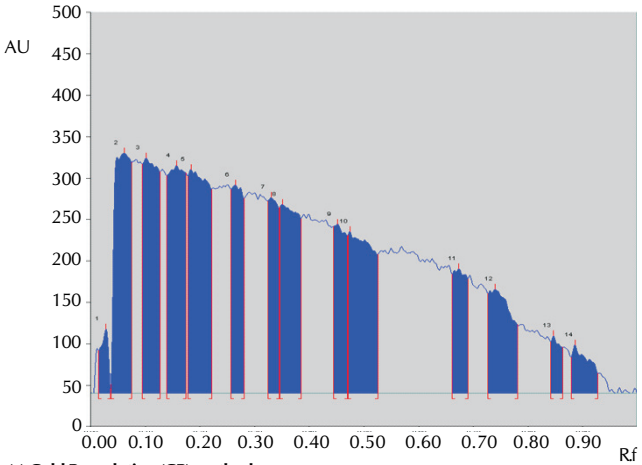


(a) Cold Percolation (CP) method

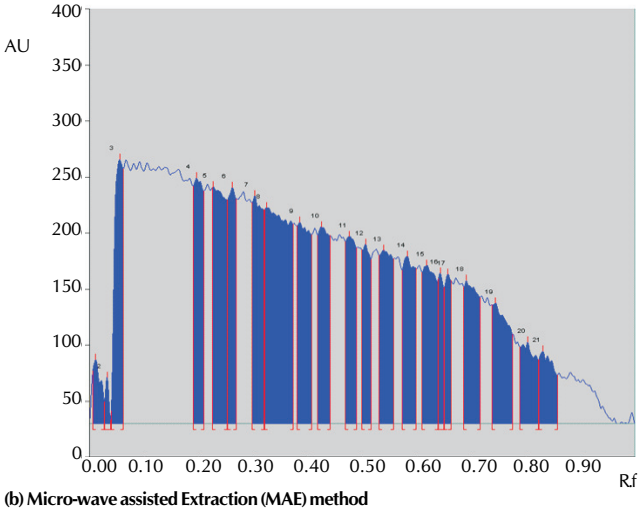


(b)Micro-wave assisted Extraction (MAE) method

Figure 2: HPTLC chromatogram of aqueous (Gr-A) extract of *Paederia foetida* shoot

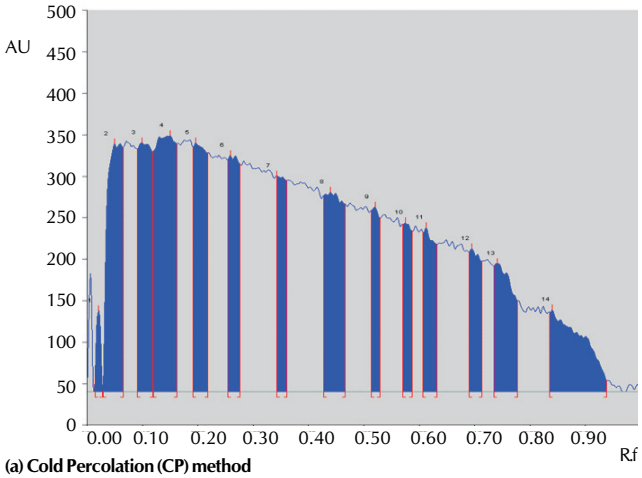


(a) Cold Percolation (CP) method

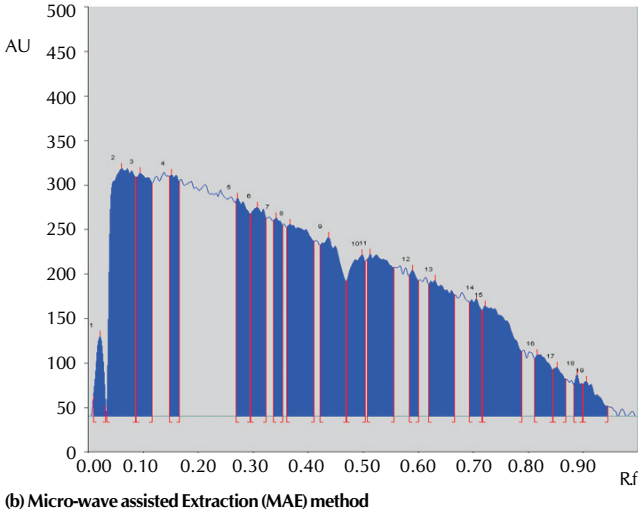


(b) Micro-wave assisted Extraction (MAE) method

Figure 3: HPTLC chromatogram of 80% methanol (Gr-B) extract of *Paederia foetida* shoot



(a) Cold Percolation (CP) method



(b) Micro-wave assisted Extraction (MAE) method

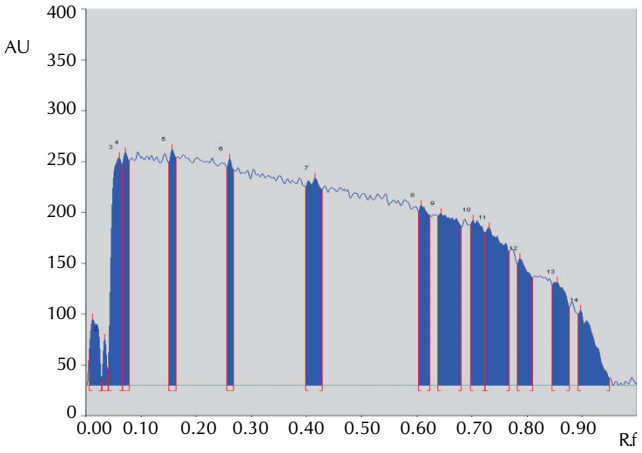
Figure 4: HPTLC chromatogram of 80% ethanol (Gr-C) extract of *Paederia foetida* shoot

0.01- 0.94), 14 (R_f 0.01- 0.95) and 16 peaks (R_f 0.01- 0.96) for Gr-A, B, C, D and E solvents (Table 4 and Fig. 2a, 3a, 4a, 5a and 6a) respectively. The peaks in respective solvent groups in MAE method (Table 5) are 15 (R_f 0.01- 0.95), 21 (R_f 0.01- 0.86), 19 (R_f 0.01- 0.94), 18 (R_f 0.01- 0.94) and 22 (R_f 0.01- 0.94) as presented in Fig. 2b, 3b, 4b, 5b and 6b. MAE method and organic solvent groups depict more number of peaks for polyphenols and flavonoids than their respective counter groups.

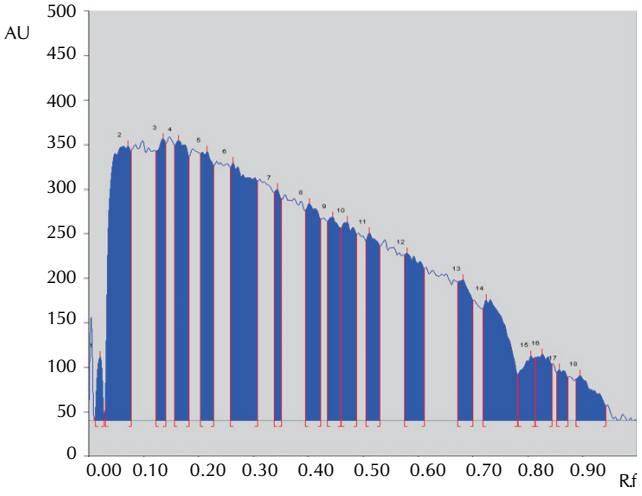
DISCUSSION

Phyto-polyphenols are secondary metabolites/ their derivatives/ isomers of flavones, isoflavones, flavonols, catechins and phenolic acids. More than 8,000 structural variants of polyphenols and 3,000 flavonoids are distributed in different parts of plants. Since, phenolic components vary in structure, physical and chemical properties, their solubility becomes different according to polarity of solvents. Most of the phenolic components are soluble in organic polar solvents for which methanol, ethanol, acetone, solvent mixture in Gr-B, C, D and E extract more principles from herb shoot and water at Gr-A being non-polar solvent fails to recover all the phenolic compounds in qualitative detection (Palanisamy and Natesan, 2012). Presence of more components in Gr-E solvent may be due to blending of polar and non-polar solvents together where the efficacy of extraction increases. Absence of certain phenolic constituents may be attributed to variation in physical and chemical behaviour of specific component in a particular solvent (Subramanian and Ramakrishnan, 2011 and Behera *et al.,* 2012a).

The polarity of solvents and physical/ chemical properties of active components play a crucial role on quantity of phenolic compounds during extraction. Method of extraction and physical conditions of exposure add to the yield. CP method involves exposure of the solvent at 10ºC for 24h. Additional effects of more temperature and pressure on physical and chemical properties of solvent is absent. So, CP method fails to recover more phenolics and flavonoids in all the polar and non-polar solvents and therefore, significantly lower ($p < 0.05$) total polyphenol and flavonoids are estimated in all the solvent extracts in comparison to MAE method. MAE method is an advanced technique of extraction of bio-active compounds and is associated with solvent type and concentration (Turkmen *et al.,* 2006, Yilmaz and Toledo, 2006). The process of extraction involves microwave energy, higher temperature

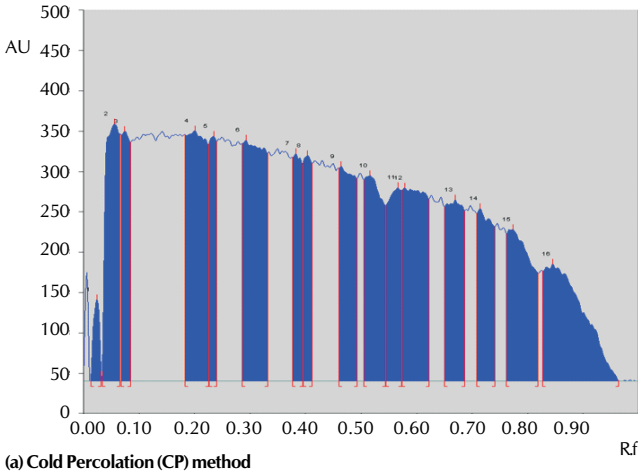


(a) Cold Percolation (CP) method

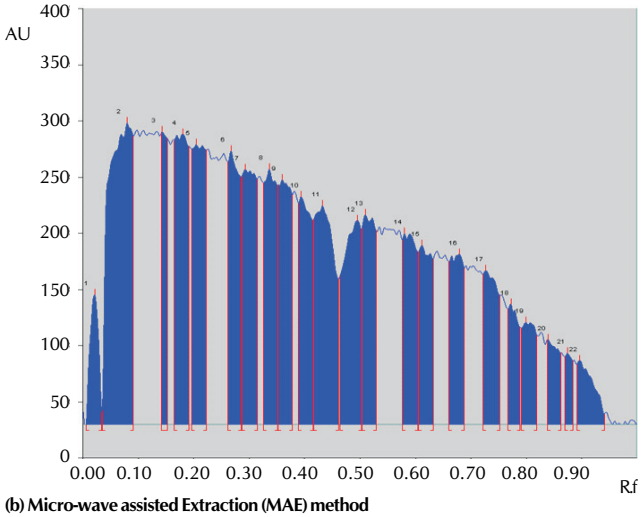


(b) Micro-wave assisted Extraction (MAE) method

Figure 5: HPTLC chromatogram of 80% acetone (Gr-D) extract of *Paederia foetida* shoot



(a) Cold Percolation (CP) method



(b) Micro-wave assisted Extraction (MAE) method

Figure 6: HPTLC chromatogram of solvent mixture (Gr-E) extract of *Paederia foetida* shoot

and pressure which intervene on the polarity of solvents. This may be attributed for estimating significantly higher ($p < 0.05$) phenolics and flavonoids in all the solvent groups by MAE method (Behera *et al.,* 2012b).

Organic solvent groups in MAE method extract significantly higher contents of phenolics and flavonoids. It exhibits more number of peaks in HPTLC analysis than the non-polar solvent and than those in CP method. Phenolic and flavonoid components like quercetin, sebiferine and litseferine are extracted under protection cover of water in 80% polar solvents. So, increased numbers of peaks in organic solvent groups are due to presence of these components. Phenolic components as glycosides/ aglycones has lower R_f values (0.00-0.25) and oligo-hydroxylated and meth-oxylated components has more R_f values (0.5-0.75). The variations in number of peaks of phenolics between solvents may be due to their occurrence with respect to R_f values. Quercetin-3-glucosides, quercetin-3-arabinosides, quercetin-3-rhamnoglucosides and Kaempferol glucoside has 0.33, 0.35, 0.29 and 0.54 R_f values respectively. The existence of least common number of peaks in all the solvents is due to occurrence of these components for their compatible R_f values observed in the study. Less number of peaks in water at Gr-A and in CP method may be attributed to low extraction/ missed phenolic components on the basis of polarity of solvent and lack of temperature and pressure effect during extraction process.

CONCLUSION

Among two methods of extraction more components of polyphenols are detected qualitatively and significantly higher contents of phenolics and flavonoids are estimated quantitatively in advanced MAE method than traditional CP method. Out of two types of solvents, organic solvent mixtures and particularly at Gr-E has greater effect on extraction for recovering significantly higher polyphenols and flavonoids in comparison to aqueous solvent. The above observations are confirmed from finger print chromatogram of HPTLC analysis.

REFERENCES

Afroz, S., Alamgir, M., Khan, M. T. H., Jabbar, S., Nahar, N. and Choudhuri, M. S. K. 2006. Antidiarrhoeal activity of the ethanol extracts of *Paederia foetida* Linn. (Rubiaceae). *J. Ethnopharmacology*. **105:** 125-130.

Behera, P. C., Bisoi, P. C. and Parija, S. C. 2012a. HPTLC detection of polyphenols and flavonoids of *Careya arborea* leaves and study of antimicrobial effect. *Int. J. Phytopharmacology*. **3(1):** 36-41.

Behera, P. C., Senapati, M. R. and Parija, S. C. 2012b. Effect of Solvents on Extraction of Certain Medicinal plant Polyphenols. *Am. J. Pharm Tech Research*. **2(6):** 428-434.

Dey, Y. N. and Pal, M. K. 2011. Evaluation of anthelmintic activity of leaves of *Paederia foetida*. *Int. J. Pharma Bio Sciences*. **2(1):** 227-231.

Eskilsson, C. S. and Bjorklund, E. 2000. Analytical-scale microwave-assisted extraction. *J. Chromatography*. **902:** 227-250.

Khan, S., Zahan, D., Das, R., Nasrin, D., Ahsan, S., Ahmed, R., Sadat, A. F. M. N., Bashar, A. B. M. A., Nahar, N. and Rahmatullah, M. 2011. Antihyperglycemic Activity Studies with Methanol Extract of *Madhuca Indica* J.F. Gmel. Leaves and *Paederia Foetida* L. Stems in Mice**.** *Advances in Natural and Applied Sciences*. **5(2):** 122-126.

Kumar, J. and Surh, Y. 2008. Cancer chemopreventive and therapeutic potential of resveratrol: Mecha-nistic perspectives. *Cancer. Lett*. **269:** 243-246.

Majumdar, T. Deb, B., Das, A., Chakraborty, A. and Goswami, B. B. 2011. Isolation of antimicrobially active compounds from the leaf of tribal edible plants of Tripura: *Momordica charantia* L. and *Paederia foetida* L. *J. Nat. Prod. Plant Resour*. **1(4):** 108-116**.**

Mandal, V., Mohan, Y. and Hemalatha, S. 2007. Microwave Assisted Extraction- An Innovative and Promising Extraction Tool for Medicinal Plant Research. *Pharmacogn. Rev*. **1(1):** 7-18.

Mimica-Dukic, N. 1992. Investigation on secondary biomolecules in some Mentha species. *Ph. D. Thesis*, University of Novisad.

Morshed, H., Islam, Md. S., Parvin, S., Ahmed, M. U., Islam, M. S., Mostofa, A. G. M. and Sayeed, M. S. B. 2012. Antimicrobial and Cytotoxic Activity of the Methanol Extract of *Paederia foetida* Linn. (Rubiaceae)**.** *J. Appl. Pharmaceu. Science*. **02(01):** 77-80.

Osman, H., Rahim, A. A., Isa, N. M. and Bakhir, N. M. 2009. Antioxidant Activity and Phenolic Content of *Paederia foetida* and *Syzygium aqueum*. *Molecules*. **14:** 970-978.

Palanisamy, H. and Natesan, R. 2012. Chromatographic finger print analysis of *Rumex vesicarius* L. by HPTLC technique. *Asian Pac. J. Trop. Biomedicine*. **2(4):** 57-63.

Reddy, K. S., Kumar, A. S. and Ganapaty, S. 2011. Evaluation of anti-ulcer activity of *Paederia foetida* root extracts in experimentally induced gastric ulcer in rats. *Int. J. Res. In Ayur. Pharmacy*. **2(5):** 1556-1559.

Saxena, H. O. and Brahman, M. 1995. The flora of Orissa. Vol. II: Orissa Forest Development Corporation Ltd., Bhubaneswar.

Senapati, M. R., Behera, P. C., Sarangi, L. N., Parija, S. C, Maity, A. and Bisoi, P. C. 2013a. Antibacterial effect of the polyphenols of *Paederia foetida* and *Artimisia nilagirica* herbs of coastal Odisha. *Anim. Sci. Repoter*. **7(1):** 3-8.

Senapati, M. R., Behera, P. C. and Bisoi, P. C. 2013b. Extraction Techniques for Phenolic Contents of *Artimisia nilagirica*. *Indian Vet. J.* **90(2):** 77-79.

Singh, R. P., Murthy, K. N. C. and Jayaprakasha, G. K. 2002. Studies on antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *J. Agric. Food Chemistry*. **50:** 81-86.

Snedecor, G. W. and Cochran, W. G. 1994. Statistical Methods, 6th ed., Oxford and IBH Publishing Co. New Delhi.

Stahl, E. 2007. Thin Layer Chromatography: A Laboratory Handbook. 2nd ed., New York: Springer-Verlag Berlin Heidelberg. 60-102.

Subramanian, S. and Ramakrishnan, N. 2011. Chromatographic finger print analysis of *Naringi crenulata* by HPTLC technique. *Asian Pac. J. Trop. Biomedicine*. **1(10):** 195-198.

Sultana, B., Anwar, F. and Ashraf, M. 2009. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*. **14:** 2167-2180.

Turkmen, N., Sari, F. and Velioglu, Y. S. 2006. Effect of extraction solvents on concentration and antioxidant activity of black and blackmate polyphenols determined by ferrous tartarate and Folin-ciocalteu methods. *Food Chemistry*. **99:** 838-841.

Uddin, B., Nahar, T., Basunia, M. A. and Hossain, S. 2011. *Paederia Foetida* Protects Liver Against Hepatotoxin-Induced Oxidative Damage. *Adv. Biol. Research*. **5(5):** 267-272.

Yilmaz, Y. and Toledo, R. T. 2006. Oxygen radical absorption capacities of grapewine industry by-products and effect of solvent type on extraction of grape seed polyphenols. *J. Food Compost. Analysis*. **19:** 41-48.