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SHORT COMMUNICATION

Microwave assisted extraction of phenols from *Paederia foetida* L. and evaluation of their antioxidant potential

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ABSTRACT

In the present investigation we have examined the comparative efficacy of four solvent systems (i.e, 80% methanol, 80% acetone, 50% acetone and mixture) and two extraction techniques (cold percolation and microwave assisted extraction) on the recovery of antioxidant principles in Paederia foetida *extracts. Appreciable amounts of total phenolic (2.22-3.98 mg GAE /g of sample), total flavonoid (0.26-1.05 mg of rutin eq/g of sample), total antioxidant (1394.23-3143.44 µM ascorbic acid eq / g of sample), FRAP (4.60-9.53 x10^3 ìM of ascorbic acid eq/g of sample), reducing power (180.23-1776.80 µM ascorbic acid eq/g of sample), superoxide radical scavenging activity (5.74-70.55%), nitric oxide scavenging action (34.25-73.16%), hydroxyl radical scavenging activity (30.46-41.36%) and percent inhibition of lipid per oxidation (23.58-41.45%) were detected in the different types of extracts. Our results suggest that (i) microwave assisted extraction (MAE) produced higher content of total phenolics and total flavonoid than cold percolation (CP) technique and; (ii) antioxidant activity of the plant extract obtained through MAE using solvent mixture of acetone, ethanol, water and acetic acid in the ratio of 40:40:19.9:0.1 v/v were significantly higher than other solvent systems or CP method.*

INTRODUCTION

New anti-oxidant principles derived from medicinal plants, are often cheaper, locally available, easily acceptable and relatively unadulterated simple preparations [26]. In majority of such phyto-preparations, however, the pure active chemical constituent(s) have not been validated scientifically with respect to their efficacy and mechanisms of action [2]. The ingestion of natural herbal antioxidants has also been shown to be associated with reduced risks of cancer, cardiovascular disease, diabetes, inflammation, bacterial disease and disorders associated with ageing [11, 27] This is perhaps owing to their free radical scavenging activities [30] via blocking the initiation or advancement of oxidizing chain reactions or by scavenging various classes of

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reactive species or chelating transition metal ions [8]. Extraction methods used to isolate phytochemicals are the major concern to enhance their recovery and efficiency [5]. Recovery of antioxidant compounds from plant materials in particular, is typically governed by the nature of extracting solvent based on their varied chemical characteristics, polarities and uneven distribution in the plant matrix [25]. Ellaborating on these lines, the present study was undertaken to standardize an efficient extraction technique for isolating anti-oxidant constituents from an Indian medicinal herb *Paederia foetida* by verifying the solevent systems during cold percolation (CP) and microwave assisted extraction (MAE) procedures.

Paederia foetida L. belonging to family Rubiaceae [28] is found in Himalayas from Dehradun eastwards upto an altitude of 1800m and also in Assam, Bihar, Odisha, and West Bengal. The plant is commonly known as Gandhali in Hindi, Shunkvine in Enligh and Pasharuni in Odia. Macroscopically, the plant leaf is 10 to 15 cm long; 5 to 6 mm width with a petiole length of 1.2 to 6 cm. The leaves are glabrous and mostly ovate having a characteristic odour and distinct bitter taste. It is reported to be used for treating gout, vesical calculi, piles, inflammation of the liver, emetic diarrhoea, dysentery [3] and to inhibit intestinal motility [1]. It is also one of the constituent in Dasmularishta , which is used in Ayurveda to treat enteromegaly, enterosis, flatulence, gastromegaly, rheumatism, rhinosis, sapraemia, sore, stomachache and toothache [10]. Ethanolic and Methanolic extract of this plant were found to be antitussive [15] with significant antioxidant activity [17]. Preliminary qualitative chemical tests showed that plant harbors a variety of carbohydrates, proteins, amino acids, tannins, phenolics, flavonoids, steroids, mucilage and saponins. [31].

MATERIALS AND METHODS

Plant material

The plant material of *Paederia foetida* L. was collected and identified in the Department of Botany, O.U.A.T. as per standard description [20]. The whole shoot was collected at pre-flowering stage, cleaned, dried under shade and ground into fine powder for preparation of extracts. A voucher specimen of the plant material was kept in herbarium for future reference.

Extraction

Extractions were carried out intro using two procedures. In trial-I, 2 g of ground mass in 20ml of solvent was heated at 80^0C for 25 minutes followed by 15 minutes cooling using Microwave Assisted Extraction (MAE) system by employing Multiwave 3000-801V (Anton Paar) digestion system [6]. In trial-II, for cold percolation (CP), 2 g tissue sample in 40 ml of solvent was kept on a magnetic stirrer at 10^0C temperature for 24 hrs followed by filtration of the extract.

The extracts were classified into four groups based on the solvents used in the two procedures such as Gr-A (80% methanol), Gr-B (80% acetone), Gr-C (50% acetone), Gr-D (solvent mixture consisting of acetone: ethanol: water: acetic acid in 40:40:19.9:0.1 v/v).

Phenolic estimation and antioxidant activity

Total polyphenol and flavonoids in the extract was determined as per reported procedure [14, 21]. The total antioxidant activity of extracts was evaluated by phosphomolybdenom [19] and reducing power [18] methods respectively. Individual antioxidant capacity of extracts was estimated by FRAP method [4] where as superoxide (SO), nitric oxide (NO) and hydroxyl radical ($OH^{i\%}$) Scavenging activities were determined by method reported by sreejayan Rao [24]. The lipid per-oxidation inhibition assay (LPOIA) was conducted accordings to the method of Ohkawa [16].

Statistical analysis

The data was subjected to analysis of variance [22] to test the significance of difference of mean values between different treatments used for extraction.

RESULTS AND DISCUSSION

Table.1 shows the comparison of total polyphenols and flavonoids contents obtained

Table.1: Comparative efficacy of different extraction technique and solvent systems on recovery of polyphenols and flavonoids from *P. foetida* and their antioxidant potential (Mean±SE).

Groups	Gr-A		Gr-B		Gr-C		Gr-D	
Methods	**MAE**	**CP**	**MAE**	**CP**	**MAE**	**CP**	**MAE**	**CP**
TP (mg of GAE/g)	3.72 [a] ± 0.60	2.22 [b] ± 0.20	3.58 [a] ± 0.50	2.76 [a] ± 0.06	3.42 [a] ± 0.45	2.47 [a] ± 0.13	3.98 [a] ± 0.54	3.10 [a] ± 0.21
TF (mg of RE/g)	0.78 [a] ± 0.06	0.46 [b] ± 0.03	0.93 [a] ± 0.06	0.52 [b] ± 0.06	0.69 [a] ± 0.06	0.26 [b] ± 0.02	1.05 [a] ± 0.04	0.71 [b] ± 0.06
TA (µM AAE/g)	2070.55[a] ± 11.34	1394.23[b] ± 13.17	2844.06[a] ± 11.83	1839.77[b] ± 4.65	1897.53[a] ± 12.60	1439.69[b] ± 10.61	3143.44[a] ± 11.80	1900.14[b] ± 14.99
FRAP (10^3x µM AAE/g)	8.32 [a] ± 1.35	4.60 [b] ± 0.29	9.17 [a] ± 1.28	5.73 [b] ± 0.09	7.70 [a] ± 1.01	4.56 [b] ± 0.05	9.53 [a] ± 1.28	6.00 [b] ± 0.38
RP (µM AAE/g)	1333.97[a] ± 9.50	467.11 [b] ± 13.17	1735.00[a] ± 8.62	180.23 [b] ± 3.01	1381.07[a] ± 13.29	194.00 [b] ± 3.00	1776.80[a] ± 15.71	269.57 [b] ± 18.64
Scavenging of SO (%)	21.84 [a] ± 1.02	16.57 [b] ± 0.55	10.95 ± 0.21	Nil	70.55 [a] ± 0.57	22.46 [b] ± 0.61	5.74 ± 0.80	Nil
Scavenging of NO (%)	61.22 [a] ± 1.81	44.21 [b] ± 2.74	58.37 [a] ± 1.37	45.90 [b] ± 3.17	73.16 [a] ± 2.30	69.30 [b] ± 1.20	53.57 [a] ± 2.79	34.25 [b] ± 0.87
Scavenging of OH^- (%)	41.09 [a] ± 0.99	40.25 [a] ± 0.64	41.35 [a] ± 0.88	30.46 [b] ± 1.30	40.83 [a] ± 0.94	35.92 [b] ± 0.87	41.36 [a] ± 0.60	39.79 [b] ± 0.72
LPOIA	31.16 [a] ± 1.17	28.33 [a] ± 0.81	31.47 [a] ± 1.43	23.58 [b] ± 0.97	41.23 [a] ± 1.15	34.66 [b] ± 1.49	41.45 [a] ± 1.36	34.94 [b] ± 1.73

Different superscripts between columns shows significant difference ($p< 0.05$) within a group. MAE-Microwave assisted extraction, CP- Cold percolation, TP- Total phenolics, GAE-Gallic acid equivalent, TF- Total flavonoid, RE- Rutin equivalent, TA- Total antioxidant, AAE- Ascorbic acid aquivalent, FRAP- Ferric reducing antioxidant power assay, RP- Reducing power, SO- Super oxide, NO- Nitric oxide, $OH^{\bullet-}$ - Hydroxyl radical, LPOIA- Lipid per-oxidation inhibition assay.

through MAE and CP methods of extraction. Total phenolics in the shoot extract of *Paederia foetida* in solvent of Gr-A was significantly higher ($p<0.05$) under MAE method in comparison to that in CP. MAE also extracted significantly higher ($p<0.05$) amount of flavonoids in all solvents. Higher amount of polyphenols in Gr-D solvents followed by Gr-A and flavonoids in Gr-D solvents followed by Gr-B were extracted in MAE method as evidenced earlier [12]. Microwave assisted extraction is an advanced technique where extraction of bio-active compounds is associated with solvent type and concentration [29]. The polarity of solvents and physical and chemical properties of components play a crucial role to affect the concentration of compounds to be extracted, but non-polar solvents are not affected by microwave energy. Polyphenols and flavonoids are more soluble in organic solvent than in aqueous solution and all the solvents contain only 20% water except Gr-C which has 50% water. Less amount of water in polar solvents protects and prevents phenolic compounds from being oxidized by phenol-oxidase [9]. Significantly higher contents of phenolics and flavonoids in G-A, B and D and lower concentrations in Gr-C may be due to variations in the solubility and difference in properties of components.

The extracted polyphenols and flavonoids from the shoot of experimental plant under MAE method exhibited significantly higher ($p<0.05$) total anti-oxidant, FRAP, reducing power, NO and SO scavenging activities in all groups of solvents than those extracted using CP method. On the other hand, the OH scavenging activity and LPOIA were significantly higher ($p<0.05$) in MAE method in all the groups except Gr-A than in CP method. Similarly, extracted components at GR-B, C and D exhibited better potency for anti-oxidant activities. The anti-oxidant activities of phenolics and

flavonoids in different methods vary between solvents where in Gr-D solvents exhibited higher total anti-oxidant activity followed by Gr-B. Similarly Gr-D solvents also depicted higher FRAP and reducing power activities followed by Gr-B solvents. SO and NO radical scavenging activity were estimated to be higher in Gr-C solvents followed by Gr-A whereas OH• scavenging property was higher in Gr-D solvents followed by Gr-B. Similarly the LPOIA was higher in Gr-D followed by Gr-C. The scavenging of superoxide, nitric oxide, hydroxyl radical and inhibition of MDA production are chemical methods to measure the antioxidative efficacy of bio-active compounds [7, 23]. Polyphenols have more than 8000 structural variants and not a single component is responsible for possessing all the anti-oxidant activities. As the components and the concentration of phenolics and flavonoids vary with the solvents and extraction methods, it contributes to their variable total and individual anti-oxidant action [23]. Microwave assisted extraction is the superior technique than other conventional methods, now a day, by adopting its standard protocols within very less time, with better extraction [13] which precisely correlates with our result.

CONCLUSION

MAE method of extraction from *Paederia foetida* recovered significantly higher ($p<0.05$) polyphenols and flavonoids than conventional method of cold percolation. Organic solvents like 80% acetone, 80% methanol and their mixture extracted significantly higher ($p<0.05$) amount and more components of polyphenols and flavonoids than 50 % acetone due to increase in polarity of solvents and better solubility of phyto-constituents. Higher concentration of phenolics and flavonoids in these solvents was reflected in their significantly higher ($p<0.05$) total and individual anti-oxidant activities in different assays.

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