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Research Article

EVALUATION OF ANTIOXIDANT PROPERTIES AND TOTAL PHENOLIC CONTENT OF MEDICINAL PLANTS USED IN DIET THERAPY DURING POSTPARTUM HEALTHCARE IN RAJASTHAN

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ABSTRACT

Childbirth is one of the most precious times in the life of a woman. Ayurveda, the traditional medicinal system of India offers a detailed diet therapy for postpartum healthcare to rejuvenate women's health. This has made the exploration for these traditional medicines and substantiates their traditional use on the ground of modern scientific pharmacological assays. The specific objectives of this study were to identify plant species used in the postpartum healthcare, to determine their total phenolic content, to characterize their antioxidant activities, and to determine if any pharmacological studies have been conducted on these plants which can be related to postpartum disorders. The investigation supports the traditional use of these plants in postpartum care.

Keywords: Antioxidants, Phenols, Postpartum care, Medicinal plants

INTRODUCTION

Reactive oxygen species (ROS) are produced as a natural byproduct/ intermediates in biological processes in body by the normal oxygen metabolism. However, during times of stress ROS levels can increase dramatically, which can result in cell damage. This cumulates into a situation known as oxidative stress. ROS stimulate the pathogenesis of many diseases like atherosclerosis, ischemic heart disease, aging, inflammation, diabetes, immunosuppression and neurodegenerative diseases etc.

Antioxidants are capable of exerting protective effects against oxidative stress in biological systems[1]. They terminate ROS chain reactions by removing free radical intermediates, and inhibit other oxidation reactions by being oxidized themselves. Antioxidant based drugs and formulations for the prevention and treatment of complex diseases like Alzheimer's disease and cancer have appeared during last three decades[2]. In current herbal drug scenario, plant derived antioxidants are gaining importance because of their potential health benefits, no toxicity and side effects over synthetic antioxidants like butylhydroxyanisole and butylhydroxytoluene (BHA and BHT, respectively). Plants may contain a wide variety of free radical scavenging molecules, such as phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids), and some other endogenous metabolites, which are rich in antioxidant activity[3]. Epidemiological and *in vitro* studies suggested that plants are major constituent in antioxidant based drugs/formulations used for the prevention of complex diseases[4].

In many Southeast Asian cultures, postpartum period is considered important from point of view of recovery, by offering a period of confinement ranging from 10 to 45 days. Ayurveda offers a detailed therapeutic route line for postpartum care (rejuvenating women's physiology). Many behavioral and dietary restrictions are followed during this period and the main emphasis is given on proper nutrition. Diet therapy includes preparations of medicinal herbs in the form of decoctions, infusions and cold extracts. The herbs given during this period are traditionally known to strengthen the body and mind and prevent disorders such as postpartum depression (PPD), body aches, insomnia, indigestion and oxidative stress[5]. This diet is also helpful for the growth and development of new born as bioactive molecules pass from mother to the child through lactation. Considering the importance of this area, the present study was conducted to determine the total phenolic content and to characterize the antioxidant activities of herbal products used in diet therapy after parturition in postpartum care (postnatal period) in Rajasthan. Antioxidant activity was done using DPPH and SOD radical scavenging assays and Ferric reducing antioxidant potential assays.

MATERIALS AND METHODS

Plant material

The plant samples mentioned in Table 1 were obtained from the medicinal plant traders and identified in the laboratory based on local names and pharmacognosy.

Chemicals

2, 2'-Diphenyl-1-picryl hydrazyl (DPPH), gallic acid, 2, 4, 6-tripyridyl-triazine (TPTZ), reduced nicotinamide adenine dinucleotide sodium salt (NADH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT) and ferric chloride were obtained from Himedia, India. All other reagents and chemicals used were of analytical grade procured from local sources.

Sample collection and extraction

A total of 37 plant samples commonly consumed in India were undertaken for study. Each sample was collected from three outlets, which were pooled and considered as single sample. Each sample was extracted and analyzed in triplicates. Powered sample (500 mg) was extracted for 4 hours at room temperature by shaking on a test tube rotator with 5 ml of 60% methanol. The sample suspensions were centrifuged at 10,000 g for 15 min at 10^0C and supernatant was filtered through Whatman no.1 filter paper and the filtrate was stored at -20^0C till analysis.

Determination of total phenolic content

The plant samples were analyzed for total phenolic content (TPC) using spectrophotometer by the method of Farkas and Kiraly, 1962 [6]. Gallic acid was used as the reference standard. All determinations were carried out in triplicate and the results are expressed as mg gallic acid equivalent per gram (mg GAE/g) of extract.

Determination of antioxidant capacity

Antioxidant activity was determined by using three methods. All the three methods can be used for solid or liquid samples and are not specific to any particular antioxidant component, but applies to the overall antioxidant capacity of the sample.

DPPH radical scavenging activity

DPPH radical scavenging activity was determined according to methods described previously[7]. This method is based on the ability

of the antioxidant to scavenge the DPPH cation radical. Percentage inhibition by the sample extract was calculated.

Superoxide radical scavenging activity (PMS/NADH System)

Superoxide radical scavenging activity was determined according to method described by Jain, Ravichandran, & Agrawal, 2008 [8]. Superoxide anions were generated using PMS/NADH system and were subsequently made to reduce NBT which yields a chromogenic product, which is measured at 560 nm. Percent inhibition was calculated.

Ferric reducing antioxidant potential (FRAP) assay

The ferric reducing power of plant extracts was determined using the method of Benzie and Strain, 1996 [9]. This method is based on the reduction of a colorless ferric complex (Fe^{3+}- tripyridyltriazine) by electron-donating antioxidants at low pH. FRAP values were expressed as m mol Fe^{2+}/g of sample. The FRAP assay does not measure thiol antioxidants, such as glutathione. It actually measures only the reducing capability based upon the Fe^{+3} ion, which is not relevant to antioxidant activity mechanistically and physiologically [10].

Statistical analysis

All the data were recorded in triplicate (n=3) and results have been expressed as mean ± standard deviation. To correlate the results obtained with different methods, a regression analysis was performed and correlation coefficients were calculated.

RESULTS

Total phenolic content (TPC)

There was a wide range of phenolic concentrations in the plant extracts analyzed as shown in Table 1. The values varied from 2.46 to 645 mg GAE /g of sample as measured by the Folin-Ciocalteau method, which represents a variation of approximately 262 fold. Four extracts showed a very high phenolic content (≥ 300 mg GAE /g): *Q. infectoria* (645 mg GAE /g), *B. monosperma* (427 mg GAE /g), *A. catechu* (393 mg GAE /g), *A. latifolia* (300 mg GAE /g). Plants like *T. chebula, S. aromaticum* and *A. marmelos* extract also showed a high phenolic content of 166, 101, and 95.33 mg GAE /g of sample, respectively. Among the selected plants, *C. nucifera* and *E. cardamomum* extracts showed a very low phenolic content (2.46 and 3.86 mg GAE /g, respectively).

Table 1: Radical scavenging capacity and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare

Plant name	Local name	Part used	Total polyphenols (mg GAE /gram)	DPPH % inhibition	SOD% inhibition	FRAP (mM Fe^{+2}/g)
Aegle marmelos (Rutaceae)	Bael	Fruits	95.33 ± 2.31	80.25 ± 1.03	54.94 ± 0.88	884 ± 5.13
Anethum graveolens (Apiaceae)	Sowa	Fruits	4.73 ± 0.23	75.53 ± 2.91	37.25 ± 1.18	62 ± 1.13
Anogeissus latifolia (Combretaceae)	Dhawra	Gum	300.0 ± 4.55	86.94 ± 2.57	84.95 ± 1.62	975 ± 6.86
Areca catechu (Arecaceae)	Supari	Seeds, nut	393.33 ± 3.77	72.71 ± 0.91	43.23 ± 0.74	1003.33 ± 9.55
Asparagus racemosus (Asparagaceae)	Shatavari,	Roots	4.13 ± 0.30	26.26 ± 0.71	32.44 ± 0.58	80 ± 1.26
Butea monosperma (Fabaceae)	Kamarkas, Palas	Gum	426.66 ± 8.31	87.17 ± 1.39	80.53 ± 1.42	79.0 ± 1.26
Cinnamomum Zeylanicum (Lauraceae)	Dalchini	Bark	58.6 ± 0.29	74.31 ± 1.53	79.00 ± 2.53	222 ± 1.98
Cocus nucifera (Arecaceae)	Nariyal	Endosperm	2.46 ± 0.12	3.14 ± 0.37	10.7 ± 0.96	03 ± 0.32
Curculigo orchioides (Amaryllidaceae)	Kali musli	Tubers	11.06 ± 0.92	30.18 ± 0.71	46.32 ± 1.22	90 ± 2.64
Curcuma amada (Zingiberaceae)	Amba-haldi	Rhizome	14.93 ± 1.06	84.88 ± 2.04	12.50 ± 0.54	74 ± 1.12
Curcuma longa (Zingiberaceae)	Haldi	Rhizome	16.66 ± 0.42	78.39 ± 1.23	24.05 ± 0.14	72 ± 1.76
Desmodium auriculata (Fabaceae)	Prista parni	Roots	49.33 ± 1.00	85.15 ± 1.49	66.66 ± 0.77	110.6 ± 2.98
Desmodium gangeticum (Fabaceae)	Shal parni	Roots	13.53 ± 0.94	85.62 ± 1.32	25.48 ± 049	208.5 ± 3.56
Elettaria cardamomum (Zingiberaceae)	Elaichi	Seeds	3.86 ± 0.12	62.33 ± 1.27	30.18 ± 0.80	02 ± 0.46
Embelia ribes (Myrsinaceae)	Vai vidang	Fruits (Berries)	14.26 ± 0.42	71.67 ± 0.81	23.29 ± 0.68	422 ± 2.15
Gmelina arborea (Lamiaceae)	Gamhar	Roots	10.26 ± 0.30	89.68 ± 1.53	44.43 ± 1.29	777 ± 2.52
Litsea glutinosa (Lauraceae)	Maida lakdi	Bark	70.0 ± 0.12	79.82 ± 0.84	82.15 ± 1.83	638 ± 7.63
Mesua ferrea (Clusiaceae)	Nagkesar	Flowers	66.66 ± 0.32	87.47 ± 0.87	46.77 ± 1.02	900 ± 3.45
Mucuna pruriens (Fabaceae)	Kaunch	Seeds	38.66 ± 1.15	89.85 ± 1.76	17.99 ± 0.42	868 ± 4.04
Myrica esculenta (Moringaceae)	Kaiphal	Bark	45.33 ± 2.04	81.27 ± 3.26	75.39 ± 0.98	800 ± 3.02

Myristica fragrans (Myristicaceae)	Jaiphal,	Seeds	12.0 ± 1.04	89.99 ± 1.78	23.18 ± 0.46	633 ± 3.60
Myristica fragrans	Javitri,	Arillus of the seed	40.66 ± 1.15	88.96 ± 1.32	14.2 ± 0.38	588 ± 3.51
Oscimum bascilicum (Lamiaceae)	Ram tulsi	Leaves	9.00 ± 0.72	63.68 ± 1.80	5.04 ± 0.14	08 ± 0.53
Piper cubeba (Piperaceae)	Sheetal Mirch	Fruits (Berries)	15.53 ± 1.85	87.47 ± 0.92	9.72 ± 0.96	485 ± 6.80
Piper longum (Piperaceae)	Piparamul	Roots	4.13 ± 0.23	45.57 ± 0.12	37.13 ± 0.98	50 ± 0.96
Piper nigrum (Piperaceae)	Kali Mirch	Dried unripe fruits (Berries)	5.13 ± 0.61	57.22 ± 1.05	31.88 ± 0.73	19 ± 0.40
Quercus infectoria (Fagaceae)	Majuphal	Galls	645.33 ± 10.2	90.20 ± 2.4	90.34 ± 0.35	1115 ± 9.29
Rubia cordifolia (Rubiaceae)	Manjistha	Roots	9.60 ± 0.87	71.98 ± 2.21	49.32 ± 0.59	75 ± 1.10
Solanum suratense (Solanaceae)	Choti kateri	Roots	4.86 ± 0.65	84.41 ± 1.21	24.9 ± 062	520 ± 2.29
Smilex chinensis (Liliaceae)	Chopchini	Roots	9.26 ± 0.81	87.36 ± 1.54	55.05 ± 0.83	55 ± 1.62
Solanum indicum (Solanaceae)	Badi kateri	Roots	14.13 ± 0.80	72.68 ± 1.27	25.11 ± 0.70	733 ± 3.60
Symplocos racemosa (Symplocaceae)	Lodh	Bark	86.0 ± 0.05	75.29 ± 0.10	63.33 ± 1.54	884 ± 4.50
Syzigium aromaticum (Myrtaceae)	Laung	Dried flower buds	101.33 ± 0.23	88.13 ± 0.58	55.05 ± 1.05	675.33 ± 5.36
Terminalia chebula (Combretaceae)	Harad	Fruits	166.0 ± 0.35	78.88 ± 0.85	84.66 ± 2.24	1009 ± 8.98
Trachyspermum ammi (Apiaceae)	Ajwain	Seeds	12.46 ± 0.98	88.39 ± 1.01	0.26 ± 0.02	955 ± 7.63
Tribulus terrestris (Zygophyllaceae)	Gokhru	Fruits	10.2 ± 0.53	82.64 ± 0.98	1.10 ± 0.06	560 ± 2.80
Vitex negundo (Verbenaceae)	Negad	Seeds	5.46 ± 0.12	53.96 ± 0.96	4.25 ± 0.10	180 ± 1.26
Zingiber officinale (Zingiberaceae)	Saunth	Rhizome	11.4 ± 0.40	87.11 ± 0.61	33.78 ± 0.53	47 ± 0.65

Radical scavenging capacity

Radical scavenging capacities were determined using DPPH, SOD and FRAP assays. Results are shown in Table 1.

DPPH radical scavenging activities

DPPH radical scavenging activities of plant extracts varied from 3.14 to 90.20%, which represents a variation of approximately 30 fold. *Q. infectori*a extract showed the highest antioxidant capacity (90.20% of DPPH inhibition), followed by *M. fragrans* seeds, *G. arborea* and *M. pruriens*, which all also showed ~ 90% of DPPH inhibition. *C. nucifera* extract showed the minimum (3.14%) DPPH inhibition. Among 38 samples investigated for antioxidant activity, 20 samples showed DPPH inhibition in the range of 80-90%.

Superoxide radical scavenging capacities

Superoxide radical scavenging capacities of plant extracts tested varied from 0.26 to 90.34 % which represents a variation of about 350 fold. *Q. infectori*a extract showed the highest antioxidant capacity (90.34 %) followed by *A. latifolia* (84.95%) and *T. chebula* (84.66%). In this assay, *T. ammi* (0.26 %) showed the lowest antioxidant potential.

Ferric reducing antioxidant potential

Ferric reducing antioxidant potential of plant extracts tested varied from 2.0 mM Fe^{+2}/g to 1115 mM Fe^{+2}/g which represent a variation of about 558 fold. Similar to the results obtained for radical scavenging assays, *Q. infectori*a (1115 mM Fe^{+2}/g), *T. chebula* (1009 mM Fe^{+2}/g), *A. catechu* (1003 mM Fe^{+2}/g) and *A. latifolia* (975 mM Fe^{+2}/g) showed very strong ferric ion reducing activities. In this assay, *E. cardamomum* (2 mM Fe^{+2}/g) and *C. nucifera* (3 mM Fe^{+2}/g) showed the lowest ferric reducing capacity.

Correlation between assays

To correlate the results obtained with the different methods, a regression analysis was performed (correlation coefficient, R). The results are shown in figures 1-6 and table 2. Maximum correlation was found between SOD and TPC (R=0.5863, Figure 6). Results of antioxidant potential as determined by different assays were also correlated. DPPH and FRAP assay gave R=0.4789 (Figure 3). The lowest correlation was found between SOD and DPPH followed by DPPH and TPC (R=0.2154 and R=0.2458 respectively).

Table 2: Correlation coefficient (R) between assays

	FRAP	SOD	DPPH
SOD	0.2993		
DPPH	0.4789	0.2154	
TPC	0.4501	0.5863	0.2458

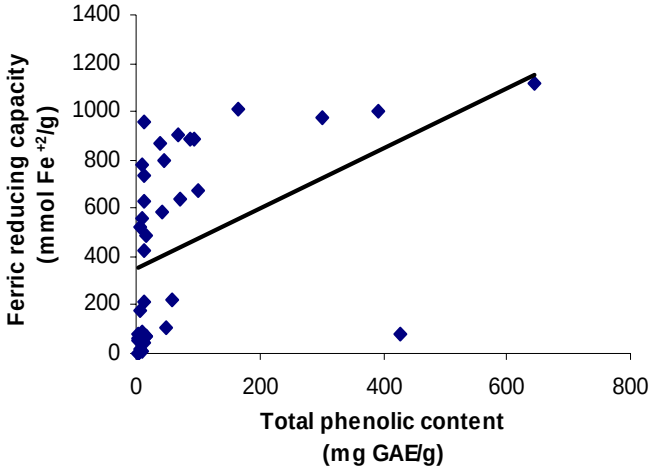

Fig. 1: Correlation between TPC and FRAP assays. Correlation coefficient R= 0.4501

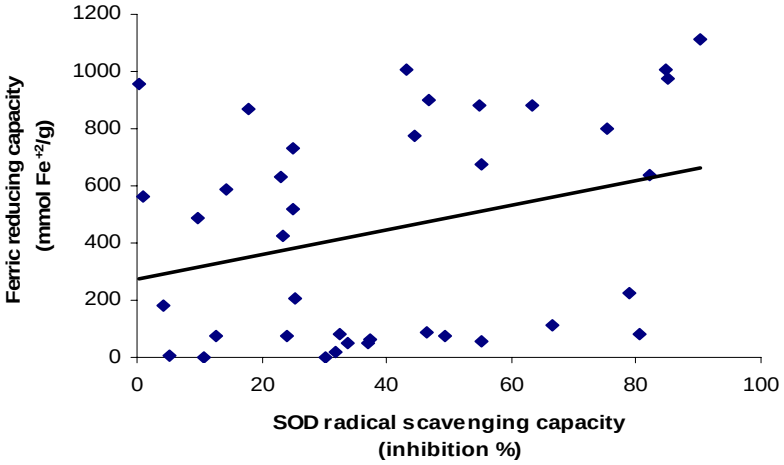

Fig. 2: Correlation between SOD and FRAP assays. Correlation coefficient R= 0.2993

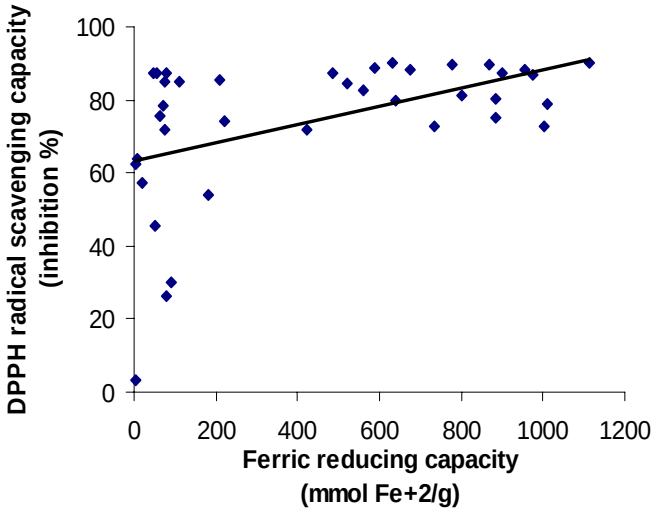

Fig. 3: Correlation between DPPH and FRAP assays. Correlation coefficient R= 0.4789

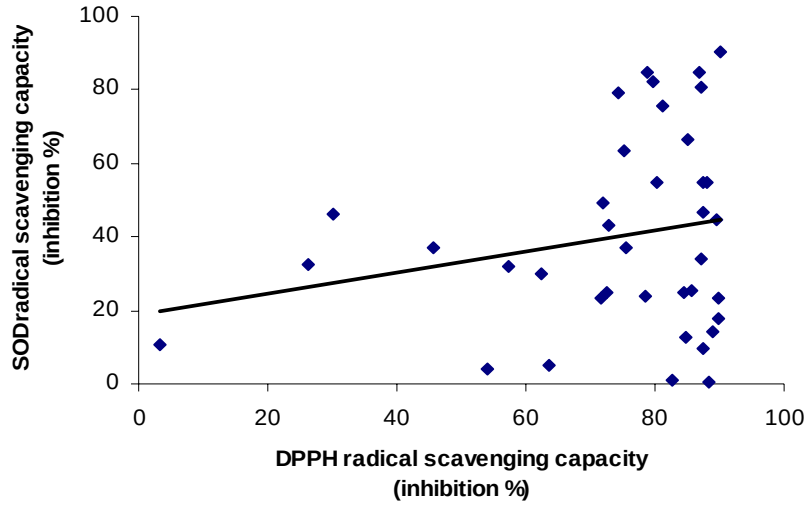

Fig. 4: Correlation between SOD and DPPH assays. Correlation coefficient R= 0.2154

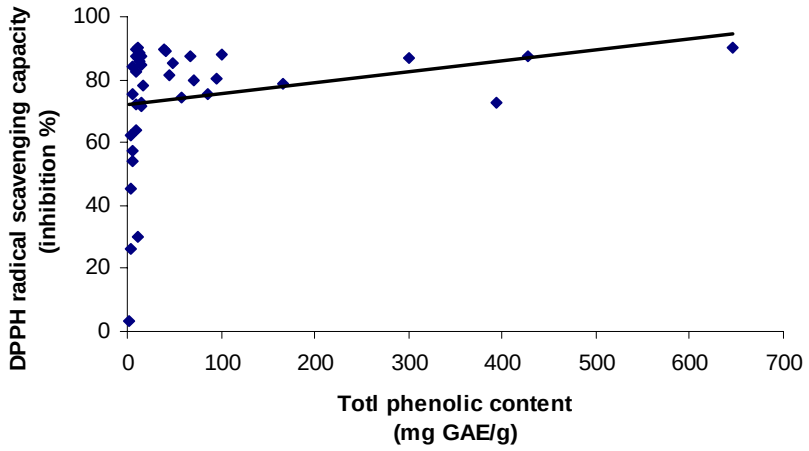

Fig. 5: Correlation between TPC and DPPH assays. Correlation coefficient R= 0.2458

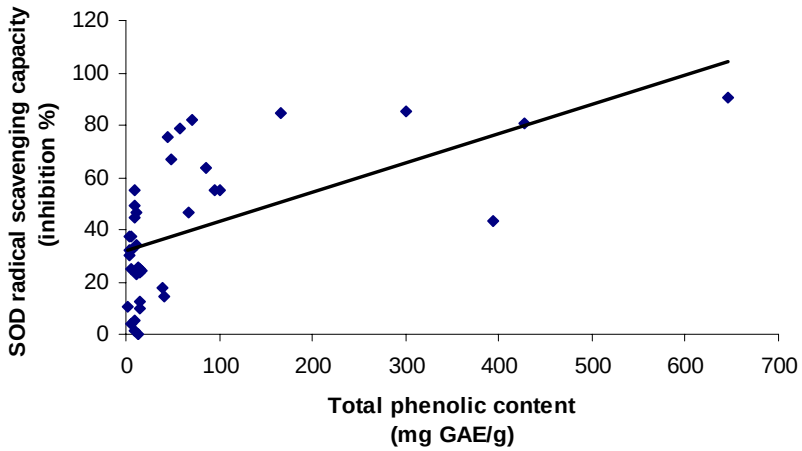

Fig. 6: Correlation between TPC and SOD assays. Correlation coefficient R= 0.5863

DISCUSSION

The greater amount of phenolic compounds leads to higher radical scavenging activity as shown by *Q. infectoria*. *Quercus* showed highest phenolic content of 645 mg GAE /gram and consequently highest FRAP, SOD and DPPH radical scavenging activity. Similarily, *T. chebula* also showed high phenolic compounds and potent SOD and FRAP radical scavenging activities. The lowest levels of phenolic compounds were recorded in *C. nucifera* and thereby the antioxidant activity as determined by DPPH and FRAP assay was also low.

In case of some plants like *G. arborea, M. fragrans, S. suratense, S. chinensis,* there was discrepancy with the total phenolic content and the antioxidant potentials found through different assays. There was no correlation between TPC and antioxidant activity. This was similar to the results observed by Mustafa et al., 2006[11] and Sreeramulu et al., 2009[7], wherein no significant correlation was observed between TPC and antioxidant activity. The lack of correlation between TPC and antioxidant capacity could be due to the fact that Folin-Ciocalteau reagent used in TPC estimation, also reacts with sugars and ascorbic acid present in plants extracts and besides them, some simple phenols also react with the reagent, although they are not effective radical scavenging antioxidants[12]. The other probable reason for antioxidant capacity does not necessarily correlate the TPC is that the molecular antioxidant responses of phenolic compounds vary remarkably, depending on their chemical structure[13].

The determination of antioxidant activity of plant extracts is an unresolved problem. The results from different antioxidant assays are even difficult to compare because of the difference in substrates, probes, reaction conditions and quantification methods[14]. There are around 20 different indices of antioxidant activity which are currently in use and no single index is considered sufficient to quantify its antioxidant activity[15].

Some plants showed lower antioxidant activities in comparison to others. Traditional use of such plants may not necessarily be related to antioxidant activities, but correlative to digestive, stimulant and galactogogue properties. In addition, these plants may also act as bioenhancers as many of them are consumed with different plants combinations.

CONCLUSION

This study showed the antioxidant properties of medicinal plants playing part significantly in postnatal recovery. The investigation supports the traditional use of these plants in postpartum care. Some medicinal plants are potent antioxidants and may be efficient as preventive agents in many diseases. The World Heath Organization has also recognized the importance of traditional medicine and has created strategies, guidelines and standards for botanical medicines. Proven agro-industrial technologies need to be applied to the cultivation and processing of medicinal plants and the manufacture of herbal medicines[16]. Therefore, the above mentioned plant species should be further explored for their other pharmacological characteristics for human welfare. Formulations can be prepared using these plants for the better healthcare of women.

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