Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2018, 10(3):340-347. DOI: 10.15835/nsb10310290
Original Article

Influence of Extraction Scheme on the Antioxidant Potential of *Caralluma tuberculata*

Sobia NOREEN[1]*, Ishtiaq HUSSAIN[1,3], Muhammad Ilyas TARIQ[1], Shahid IQBAL[1], Fozia BATOOL[1], Shazia Akram GHUMMAN[2], Shazia NOUREEN[1], Tusneem KAUSAR[4]

[1]*University of Sargodha, Department of Chemistry, Sargodha 40100, Pakistan; sobianoreen@uos.edu.pk (*corresponding author); tariqmi200@yahoo.com; ranashahid313@gmail.com; fozia.batool@uos.edu.pk; shazianoureen11@gmail.com*
[2]*University of Sargodha, Faculty of Pharmacy, Sargodha 40100, Pakistan; shaziaghumman33@gmail.com*
[3]*University of Lahore, Lahore 5400, Pakistan; h_hussain1@yahoo.com*
[4]*University of Sargodha, Department of Food Science and Technology, Sargodha 40100, Pakistan; tusneemft@yahoo.com*

Abstract

Herbal industry of developing countries is facing several technical issues related to the extraction conditions in order to attain the maximum yield of a plant extract with maximum therapeutic attributes. Therefore the present study was aimed to investigate the best technique for respective herbal products. The effect of three different extraction techniques: ultrasonic assisted extraction, microwave assisted and orbital shaker assisted extraction, by using three solvents (80% methanol, ethanol, and ethyl acetate) on the antioxidant potential of *Caralluma tuberculata* stem (non-conventional vegetable) extracts were investigated. Folin-Ciocalteu method was applied on tested samples in order to find the concentration of total phenols. Therefore, the optimized extract of high yield and maximum total phenolic content was selected for further analysis like total flavonoid contents, FRAP, DPPH and ABTS scavenging potential. Quantification of phenolic acids in the extracts was also carried out by HPLC. Significant variation was observed in the yield of total phenols within the extracts, but better results were obtained in aqueous methanolic extract of ultrasonic assisted extraction, followed by microwave assisted and orbital shaker assisted extraction. Present findings supported the view that ultrasonic assisted extraction can be used for phytochemicals profiling, activity guided assays and the development of herbal products. Correlation coefficients of active principles indicated a significant relationship to antioxidant capacity $P < 0.05$.

Keywords: DPPH scavenging potential; microwave assisted extraction; phenolic; total flavonoid contents; ultrasonic assisted extraction

Introduction

Oxidative stress arises as a result of inequality between the generation of free radicals, reactive oxygen species and scavenging action of built-in antioxidants present in the body (Ahmad, 1995; Mitra *et al*., 2010). These species are generated as toxic byproducts of different endogenous processes like aerobic metabolism, hypersensitivity, inflammation, phagocytosis, xenobiotic reduction, as well as exogenous progressions like the effect of various mutagenic chemicals, pollutants, cosmic and ultraviolet radiations occurring in body in presence of oxygen (Zima *et al*., 2001). Oxidative stress or excessive production of free radicals by the body upshots the course of aging, which is the greatest risk for variable disorders and number of serious diseases (Chen *et al*., 2013). Such type of degenerations may include atherosclerosis, arthritis, cataract, cancer, hemorrhage, hypertension, obesity, chronic inflammation, neurodegeneration, post-ischemic perfusion injury myocardial infarction and cardiovascular problems caused either by cell death or by losing the membrane integrity due to peroxidation of lipids or by deterioration or alteration of structure and functions of proteins like ion channels, enzymes and porin or mutation in DNA strand (Buttke and Sandstrom, 1994; Parr and Bolwell, 2000; Valko, 2006; Dhanani *et al*., 2017; Furukawa *et al*., 2017). Furthermore, specifically free radicals of nitrogen combined with tyrosine

Received: 04 May 2018. Received in revised form: 16 Aug 2018. Accepted: 18 Sep 2018. Published online: 27 Sep 2018.

are required for the action of ribonucleoside diphosphate reductase and eventually causes cellular toxicity (Al-Faifi *et al*., 2016). Antioxidants acts against the fabrication of free radicals through various possible ways, either by the inhibition of certain enzyme or chelation of metal ions like iron and copper etc., scavenging to reactive nitrogen (RNS) and reactive oxygen species (ROS) (Uttara*et al*., 2009; Jyoti *et al*., 2015; Kommidi *et al*., 2016). In this way, antioxidants act as safeguard against oxidative damage and prevent life from various life-threatening diseases (Liede-Schumann *et al*., 2005).

Recent era is the witness to continuous increasing requirement and consumption of antioxidants both from plants and of synthetic origin, in order to minimize the severe effects of oxidative stress. Plant-based or natural antioxidants are going to superimpose the importance of their synthetic counterparts owing to involvement of later in initiation and propagation of a number of chronic disease (Adnan *et al*., 2014). Plants contain a flurry of antioxidants in the form of phenolics, like phenolic acids, coumarins, flavonoids, lignins, stilbenes, quinones, tannins etc, nitrogen bearing compounds like amines, alkaloids, terpenoids, like carotenoids, vitamins and various other active principals. This versatile collection is exhibiting the deciphering role in oxidative stress and related dreadful diseases and disorders in this scenario. Remarkable examples of antioxidants often procured from diet are Vitamins (C and E), β-carotene and coenzyme Q (Farouk*et al*., 2016). These natural sources are safer, have a lower cost and are more effective as compared to synthetic odds, which lead to exponential rise in demand of natural antioxidants in the form of food supplements or dietary items. Numbers of cereals, legumes, spices, herbs, leaves, seeds, fruits and agro-wastes have been explored successfully. However, overwhelming demand of natural antioxidants suggests exploration of newer and newer botanical materials with promising antioxidant attributes.

Soon-sakaser valley of district Khushab, Punjab, Pakistan is the house of 6,000 rare medicinal plants owing to its classic agro climatic environment (Ahmad *et al*., 2008). *Cralluma tubercalata* (CT) is one of the wildly grown herbaceous succulent of this area and is the member of Asclepiadaceae family that comprises 424 genera (Liede-Schumann *et al*., 2005). It is consumed as non-conventional vegetable and was also reported famine food in history. This perennial leafless herb is famous for its therapeutic roles (Adnan *et al*., 2014; Jyoti *et al*., 2015). CT has been reported for its anti-inflammatory and strong hypoglycemic effect (Ahmad *et al*., 1988; Ahmad *et al*., 1993; Mahmood *et al*., 2010). In medical folklore, this is also reputable for the treatment of rheumatoid arthritis, paralysis and fever (Khan and Khatoon, 2008). All the members *Caralluma* family are rich in pregnane, that is naturally conjugated with glycoside. These molecules appear as potential lead for drug development especially against cancer, hepatitis and diabetes (Deepak *et al*., 1998; Al-Faifi *et al*., 2016; Kommidi *et al*., 2016). Pregnane glycosides series have been extracted from its organic extracts that explicit cytotoxic effect on cell line of human diploid embryonic cell like MRC5 (Abdel-Sattar *et al*., 2008; Abdel-Sattar *et al*., 2011). Other species of this family are famous for the isolation of antioxidants, whereas plant material depends upon the extraction scheme and solvent polarity according to the chemistry of principle components (Li *et al*., 2009). Epidermal and sub-epidermal layers of seeds and fruits are rich in soluble phenolics as compare to other tissues (Antolovich *et al*., 2000; Chen *et al*., 2013). Polar solvents are mostly reported as the best choice for extraction of antioxidants, while some studies proved ethyl acetate as a suitable alternate (Peschel *et al*., 2006; Sultana *et al*., 2007).

In continuation of ongoing trend, effect of different extraction procedures for the best yield of potential natural antioxidant of CT has not been studied yet. Therefore the present study was planned to disclose the convincing role of three extraction systems (sonication, microwave assisted and orbital shaker extraction) and solvents on the yield and activities of antioxidants.

Materials and Methods

Collection, identification and preservation of plant material

CT whole plant samples were collected from the Soon-sakaser valley, Khusab district, Punjab, Pakistan, during September to November 2016. Sampling area is notified on the map of Pakistan in Fig. 1.

After taxonomic identification, authenticated voucher specimens were deposited at Herbarium, University of Agriculture, Faisalabad. Freshly collected plants were rinsed with tap water and then with deionized water to make them free from dust and any metal contamination. After drying of water, roots were separated manually from the stems and stems were chopped to small pieces, followed by drying under shade at ambient conditions till the attainment of constant weight. Air dried stem samples were pulverized with electric grinder into fine coarse powder, followed by storage in polyethylene air-sealed bags for future analyses.

Chemicals

All the chemicals Folin-Ciocalteu reagent, gallic acid, 2, 2′-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid used in different experiments were purchased from the local distributor of Sigma-Aldrich Chemical Corporation Germany. While all other analytical grade chemicals: acetic acid, ethanol, methanol sodium nitrite, sodium hydroxide, ammonium thiocyanate, ferrous chloride, anhydrous

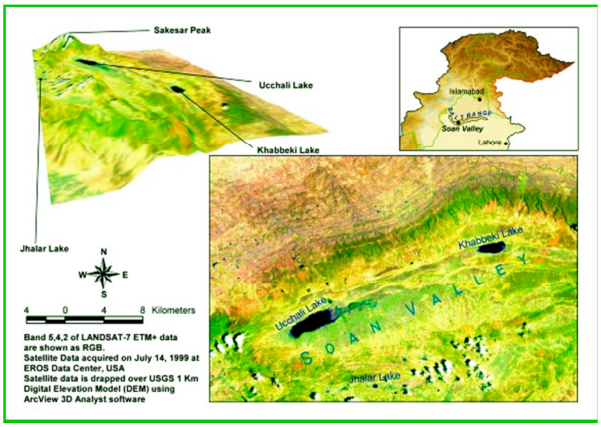

Fig. 1. Location of Soon-sakaser valley in the map of Pakistan

sodium carbonate, potassium iodide, potassium dihydrogen phosphate, aluminum chloride, dipotassium hydrogen phosphate and sodium thiosulphate used in the study were purchased from local distributer of Merck (Darmstadt, Germany) in Pakistan, unless stated otherwise.

Extraction procedures

Air ground (Mesh 80) extracts from CT stem (20 g each sample) was extracted by three different extraction schemes, ultrasonic assisted extraction (UAE), microwave assisted (MAE) and orbital shaker assisted extraction (OAE) using three solvents (80% methanol (Met_{aq}), ethanol (Eth) and ethylacetate (ETOAc), according to the reported method with little modification (Mitra *et al*., 2010). UAE extraction was carried out at medium speed of sound wave i.e. 17 kHz for 3 h, MAE was done at power of 90 W for 15 min, while OAE at speed of 150 for 3 h. All the extracts were separated from the residues at room temperature by using Whatman no. 1 filter paper. Three successive extractions were carried out by using the same fresh solvent and extract combined. The combined extract was concentrated and made free from solvents by using rotary evaporator under reduced pressure at 45 °C. Dried crude extracts were weighed, packed in labelled vials and stored at -4 °C for future analysis. The % yield of each extract was also calculated.

Determination of total phenolic content (TPC)

Extracts were subjected to Folin-Ciocalteu reagent based spectrophotometric assay for the determination of TPC following a previously reported method. Briefly, freshly prepared diluted solution of 0.1 ml Folin-Ciocalteu reagent (0.5 N) was added in extract (0.5 mL) and resulting mixture was diluted in water with ratio (2:1 g mL^{-1}) followed by incubation for 15 min and adding 7.0% Na_2CO_3 solution (2.5 mL) and subsequent incubation in dark for 30 min to let the reaction complete. Absorbance of final mixture was recorded at 700 nm using UV/Visible Spectrophotometer (CECIL CE-7200). All the measurements were conducted thrice and TPC was calculated as Gallic Acid Equivalent (mg g^{-1} dry weight) and results were computed as average (Chen *et al*., 2013).

Determination of total flavonoid contents (TFC)

Total flavonoid content of prepared extracts was determined following a method of Dewanto *et al*. (2002), with slight modifications. Briefly, 1.00 mL plant extract aqueous solution (10 mg ml^{-1}) was taken in a test tube followed by the addition of freshly prepared 0.3 mL of $NaNO_2$ solution. Then 0.3 mL of fresh $AlCl_3$ (1:10) was added in mixture after 5 min. Then after six minutes, 1.00 M NaOH (2 mL) was added and finally distilled water was added to make the 10.0 mL volume of the mixture. Absorbance was measured at 510 nm immediately. TFC of the samples was revealed as epicatechin equivalents (mg g^{-1}) of the dry weight. Test was run in triplicates and results were standardized (Valko, 2006).

2,2′-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

DPPH radical scavenging activity of CT extracts was determined according to the assay proclaimed by Proestos *et al*. (2013) with minor amendments (Dhanani *et al*., 2017). DPPH radical exhibits λ_{max} at 517 nm and potential of phyto extract for reduction in absorbance was recorded as its radical scavenging power. 0.2mM DPPH (2.00 mL) was added into 2.00 mL of methanolic solution of plant extracts (5:1) by methanol as positive control. The mixture was placed in the dark for 1 hour and the absorbance was measured at 517 nm using methanol as blank. All measurements were done in triplicate and percent inhibition of DPPH radical was calculated with the help of following relation:

$$\% \text{ Inhibition} = A_{control}-A_{sample}/A_{control} \times 100$$

50% Inhibition concentration (IC50) was calculated from the graph of scavenging activity that was plotted by using ascorbic acid as standard.

2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging assay

ABTS radical cation scavenging activity was evaluated following a modified method reported by Proestos *et al*. (2013). In brief, ABTS (7.00 mM) and solution of MnO_2 (2.45mM) were separately prepared and mixed with 1:1 ratio and incubate in dark at room temperature for 48 h. Then ABTS solution (2.00 mL) which was first diluted in methanol with ratio of 1:25, was added in 200 µL of plant extract and measured the absorbance at 734 nm. Percentage scavenging for ABTS radical cation by extract was calculated by means of the standard calibration curve. All measurements were done thrice and results were averaged (Dhanani *et al*., 2017).

Ferric reducing antioxidant power

CT extract (10 mg) and 1% potassium ferricyanide (2.00 mL) were added in 3.5 mL phosphate buffer (0.2 M, pH 6.6), followed by incubation at room temperature for 20 min. Afterward, 10% trichloroacetic acid (2.5 mL) was added into the mixture and centrifuged at 3,000 rpm for 10 min. After that, supernatant (2.5 mL) was separated and diluted with distilled water (2.5 mL). Then 0.1% ferric chloride was added in the reaction mixture. The absorbance was taken at 700 nm. Results were expressed as ascorbic acid equivalent mg g^{-1} dry weight (Furukawa *et al*., 2017).

Identification and quantification of phenolic acids by high performance liquid chromatography (HPLC)

HPLC analysis was carried out for quantification and identification of phenolic acids according to the method of Sultana and Anwar (2008). Acid hydrolysis of extract was done by 5.00 gm of CT_M extract in 50 mL methanol and 5.00 mL of 2 M HCl. The reaction mixture was refluxed for one hour in order to attain the free phenolic acids of extract. Afterward, filtered the extracts by using Whatman filter paper no. 1 and then immediately passed through 0.2 µ syringe filter. A series of commercial standards (Sigma Chemicals Co., St Louis, MO, USA) of phenolics solution was also run prior to sample run for generation of calibration curves. Analytical system of HPLC (Shimadzu, model LC-10A, Kyoto, Japan) along with UV-Vis detector (SPD-10AUV λmax 360nm), CTO-10A column oven, Rheodyne injector and LC-10 AS pumps. At ambient temperature separation was achieved in Shim-Pack CLC-

ODS (C-18) column (4.6X250 mm, i.d. 5µm). Solvent employed were A (H_2O:acetic acid 94:6, pH2.27), B (acetonitirle 100%). The gradient profile was 0-15 min = 45% B, 30-45 = 100% B with flow rate of 1.00 mL/min. UV/Visible detector was set at 280 nm for quantitative determination. Phenolic acids of sample extract were identified and quantified by comparing the retention time to the standards.

Statistical analysis and data presentation

All antioxidant assays were performed thrice from the same extract in order to confirm its reproducibility. Statistical interpretations of experimental data (all in triplicate) were articulated as mean ± standard deviation. Analysis of variance was used used to check the liability of sample antioxidant activities resulting from these methods. Results were executed in graph particular via GraphPad Prism version 5.00 for Windows, San Diego California USA and Minitab 17. Correlations between data obtained were calculated using Pearson's correlation coefficient (r) in SPSS Statistics V21.

Results and Discussion

The influence of different extraction procedures and solvents on the percent yield and antioxidant potential was investigated. All dried extracts were dark green in colour and their percent yield was high in case of aqueous methanolic extract obtained by UAE protocol i.e. 17.08%. Meanwhile, the lowest yield of 9.25% was obtained in the ethylacetate extract obtained by OAE. The 3-D response surface models (RSM) are considered very valuable for understanding the significant effect of independent variables (extraction scheme and solvent types) upon dependant factors (extract yield and total phenolic contents). Surface plot of percent yields in order to find the optimum method is shown in Fig. 2A.

The reason for high yield of UAE is that ultrasonic waves of medium speed disrupt the plant cell boundaries, as result internal material ooze out and thus increasing the surface area for mass transfer of solute (Ameer *et al*., 2017; Furukawa *et al*., 2017). Though the percentage yield in the case of MAE was also good enough, but recovery was plateau (Antolovich *et al*., 2000).

Total phenolic contents (TPC)

Phenolics are considered therapeutically active constituent that deciphers a wide range of activities like anticancer, antiviral, antibacterial, antifungal, anti-allergic etc. Phenolic compounds are produced in plants in response to various factors like UV radiation, stress like drought, nutrition deficit, harsh temperature and pressure condition and defensive action against pathogens. Total phenolic contents of different solvent fractions of CT by three different techniques determined by a modified Folin-Ciocalteu method and results were expressed in graphical forms (Fig. 2B and Fig. 3). TPC were resolute in the hereby study weighed against with standard (gallic acid) and final results are articulated in term of milligrams of gallic acid equivalent (mg GAE g^{-1} dry sample).

Among three solvent extracts, aqueous methanolic and ethanolic extract offered the maximum concentration of TPC i.e. 70.68± 0.5, obtained by UAE, followed by arbitrary response to Eth. 46.35± 0.4 and EtOAc 34.01± 0.5 fractions of other two extraction approaches. This fact is justifiable by the fact that polar phenolics concentration is high in polar solvents as compared to non-polar solvent (Uttara *et al*., 2009).

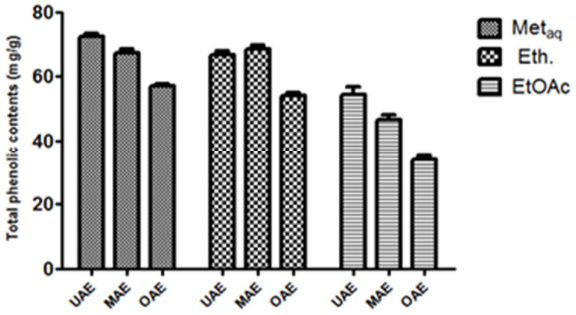

Fig. 3. Effects of different solvents and techniques on the total phenolic contents of CT extracts (GAE mg g^{-1} of dried Sample); UAE: ultrasonic assisted extraction, MAE: microwave assisted, OAE: orbital shaker assisted extraction, Met$_{aq}$: aqueous methanol, Eth: Ethanol, ETOAc: Ethylacetate. Values are average (n = 1x3) and represented as mean ± SD.

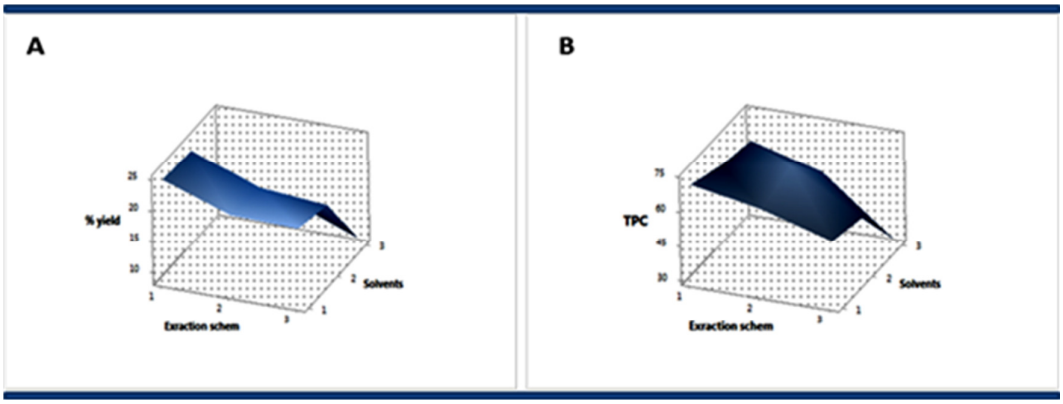

Fig. 2. Response surface plot, indicating the joint effect of solvent types and extraction scheme on maximum yield (A) and total phenolic contents (B); Independent variables are expressed in codified digits. (Extraction scheme code; 1: UAE, 2: MAE and 3: OAE solvent codes 1: Met$_{aq}$, 2: Eth and 3: ETOAc).

Hence the percent yield and TPC data of all schemes confirmed that aqueous methanolic extracts was found to be the best extraction solvent for phenols and was selected for further analysis of the present study. Reduced concentration of TPC in MAE extracts are due to the thermal degradation of phenolic compounds effect their antioxidant potential. Extraction scheme plays a deciphering role in the concentration phyto-constituents and their antioxidant potential (Dhanani *et al*., 2017).

Total flavonoids content

Flavonoids being phenolic glycosides offer antioxidant bustle of plant due to their metal chelating and scavenging power, hence act to manage patho physiology allied to oxidative stress. Structure of flavonoid contains a central backbone of three rings and sometime occurs naturally in conjugation with a glycone moiety. Oligomeric form of flavonoid is Proanthocyanidin. Due to the presence of phenolic group they both operate like antioxidant, phytoestrogen, reduce the risk of inflammation and cancer (Proestos *et al*., 2013; Ameer *et al*., 2017). Flavonoids concentration of samples CT is present in the order of 53.82 > 42.38 > 26.24 presented as mg g^{-1} Catechin equivalents the highest in sonication extract and lowest in shaker. The projected flavonoids content of CT extracts are expressed as mg g^{-1} Catechin equivalents in Fig. 4.

Free radical scavenging activity of extracts by DPPH, ABTS and FRAP assays

H-ion-donating ability of polyphenols is a vital attribute that can convert potentially toxic reactive oxygen species into the nontoxic one. Particularly, DPPH• appears quick in action in determining antioxidant potential for rapid switch to labile hydrogen atom into radicals. The absorption of purple colour solution that is formed into DPPH was taken at wavelength 517 nm. Ascorbic acid curve was used as a standard, while BHT was used as positive control. Deep colour of solution fades due to the presence of antioxidants. DPPH assay results were expressed as ascorbic acid equivalent per gram dry weight of CT extracts. Their anti-radical potential for DPPH and IC_{50} values are expressed in Table1**.** The overall trend was in the following order: BHT> UAE> MAE> OAE. The least

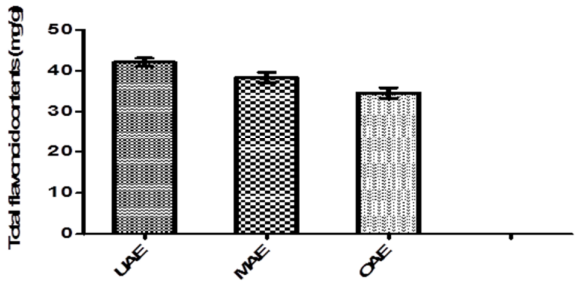

Fig. 4. Effects techniques on the total flavonid contents of CT extracts (GAE mg g^{-1} of dried sample); UAE: ultrasonic assisted extraction, MAE: microwave assisted, OAE: orbital shaker assisted extraction, Met_{aq}: aqueous methanol, Eth: Ethanol, ETOAc: Ethylacetate.Values are average (n = 1x3) and represented as mean ± SD.

DPPH scavenging potential was exhibited by CT extracts obtained by OAE scheme. The IC_{50} of UAE, MAE and OAE methanolic extract was found as 608.50, 480.20 and 387.60 μg mL^{-1} and thus UAE offer better reducing potential of DPPH.

ABTS radical scavenging expressed as ascorbic acid equaling per gram dry extract is shown in Table 1 that is varying from 262.9 μmol g^{-1} to 378.1μmol g^{-1}. The strongest IC_{50} 86.2 μg mL^{-1} was exhibited by UAE extract that is as good as to positive control BHT i.e. 83.8 μg mL^{-1}.

Among all the assays use for determination of antioxidant potential for phyto extract, the FRAP assay is the most simple, fast, inexpensive, reproducible and highly reliable method (Benzie and Strain, 1996). Antioxidant compounds can effectively reduce Fe (III) to Fe (II) exhibiting high reducing power. The reducing power is directly linked with concentration of extract that is increased when concentration rose. Ferric reducing antioxidant power of selected plant extracts are shown in Table 1. Reducing properties are due to the electron donating potential for phyto extract specific compounds (Dewanto *et al*., 2002). Table 1 show that all extracts from CT exhibited some degree of electron donating potential and reduce ferric ions into ferrous ions.

Correlation between compounds yield and antioxidant potential

Antioxidant potential for phenolic compound has been determined by their electron donating capacity and the formation of stable free radical intermediates. The present study pointed out that there is a strong correlation between the concentration of total phenolics and various antioxidant assays (Table 2). Pearson correlation results to decipher the strong correlation between yield with TPC $r = 0.999$, $P < 0.05$, DPPH $r = 0.998$, $P < 0.05$ and ABTS $r = -0.999$, $P < 0.05$. High positive correlation between yield and TPC strengthen the fact that due to hydrophilic nature phenolic are the most abundant secondary metabolites (Thaipong *et al*., 2006).TPC has a highly positive correlation with DPPH assay ($r = 0.999$, $P < 0.05$), which indicates the fact that phenolic components are mostly responsible for antioxidants capacity in DPPH method (Clarke *et al*., 2013).

There is a strong and negative relationship between TPC and ABTS which predicts that constituents repose for the assay are decreasing as the amount of TPC increases. Correlation coefficient value of TFC and FRAP ($r = 0.999$, $P < 0.05$) unveil the fact that flavonoids are the major contributors of antioxidants in FRAP. Over all view that ABTS assay has a different behaviour showing a negative correlation between yield, TPC, DPPH and FRAP.

Quantitative estimation of phenolic acid by HPLC

The extraction of phenolics from the plant cell matrix is greatly influenced by extraction protocol, nature of chemical and solvent, particle size of sample and interfering moieties etc. Identification and quantification of phenolic acids in optimized methanolic extracts of CT was done by HPLC with UV/DAD. Identification was done on the basis of retention time in comparison with the standard curves obtained after run of 12 authentic standard polyphenolics compounds (apigenin, ascorbic acid, caffeic acid, cinamic

acid, ferulic acid, gallic acid, kaempferol, myricetin, syringic acid, p-coumaric acid, quercetin, and sinapic acid) at 280 nm. Fig. 5 represents the quantification data onto phenolic acids in CT_M. Sinapic acid 6.16± 0.03 mg g^{-1} was the dominant phenol, followed by p-coumaric acid 4.62.± 0.05 mg g^{-1}, gallic acid 4.39± 0.03 mg $g^{-1}$and quercetin 0.39± 0.07 mg g^{-1}.

At the present moment, no data is available on CT extracts, but in another species of this genus (*Caralluma arabica*) different extracts like epicatechin, vanallic acid, rutin, gallic acid and quercetin were reported in various concentrations ranging from 2.43 to 0.32 mg $g^{-1}$of extract (Khasawneh *et al*., 2014). Naturally occurring sinapic acid, p-coumaric acid, gallic acid and their derivatives are thought to have a momentous role against infections, oxidative stress, cancer, diabetes and neuro-degeneration etc. (Ow and Stupans, 2003; Lou *et al*., 2012; Chen, 2015). These outcomes recommend that antioxidant potential for CT may credit to its different pharmacological properties like anticancer, antimicrobial and anti-inflammatory etc. These are beneficial to some extent for plants itself because various processes like plant immunity, cell signaling and pollination.

Table 1. Free radical scavenging power of antioxidants of $Meth_{aq}$ extracts obtained by different extraction scheme by DPPH, ABTS and FRAP assay

Sr. No.	Extraction scheme	DPPH assay		ABTS assay		FRAP assay
		TAC (µmol/g)	IC_{50} (µg/mL)	TAC (µmol/g)	IC_{50} (µg/mL)	TAC (µmol/g)
1	UAE	34.67	608.5	378.1	86.2	149.22
2	MAE	77.47	480.2	243.8	139.8	116.34
3	OAE	65.29	387.6	262.9	197.4	75.15

UAE: ultrasonic assisted extraction, MAE: microwave assisted, OAE: orbital shaker assisted extraction, Met_{aq}: aqueous methanol.*All values in table are average of three values obtained after the analysis of sample in triplicate (n = 1x3) and represented as (mean ± SD).

Table 2. Pearson correlation coefficient (r) of extraction scheme, total phenolics, total flavonoids with different antioxidant indicators

Trait	Yield	TPC	TFC	DPPH	FRAP	ABTS
TPC	0.999*					
TFC	0.992	0.989				
DPPH	0.998*	0.999*	0.982			
FRAP	0.996	0.993	0.999*	0.998		
ABTS	-0.999*	-0.997*	-0.997*	-0.994	-0.999*	

TPC= total phenolic contents, TFC= total flavonoid contents, DPPH= IC_{50} (µg mL^{-1}) values of extract based on DPPH assay, ABTS= IC_{50} (µg mL^{-1}) values of extract based on ABTS assay, FRAP=Ferric ion reduction potential in term of TAC (µmol g^{-1}) and * = significant at P< 0.05.

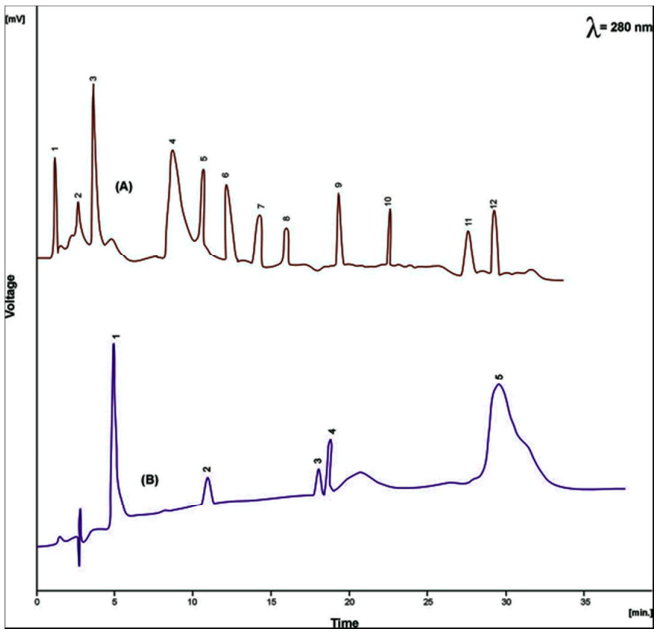

Fig. 5. Identification and quantification of phenolic acids (ppm) by HPLC. HPLC chromatogram of phenolic acid standards. Standard phenolic acid used are denoted by numbers on peak as fellow: 1. Ascorbic acid, 2. Gallic acid, 3. Caffeic acid, 4. Syringic acid, 5. p-coumaric acid, 6. Sinapic acid, 7. Ferulic acid, 8. Cinamic acid, 9. Myricetin, 10. Quercetin, 11. Apigenin, 12. Kaempferol. HPLC chromatogram for identification and quantification of phenolic acids in methanolic extract of *Caralluma tuberculata* obtained by ultrasonic assisted extraction scheme [Gallic acid (1), Caffeic acid (2) p-coumaric acid (3), Sinapic acid (4), Quercetin (5)].

Conclusions

Phenolics as phyto-constituents have attained significant position in therapeutics owing to their potential allied with health benefits, specifically oxidative stress and other lifestyle malady. The results of the present study clearly indicate that *Caralluma tuberculata* revealed a significant concentration of active antioxidant compounds in different extraction procedures. Nine experimental runs in which the effect of two independent variables (3 levels of each) on dependent variables (percent yield and total phenolic content) were performed. All experiments were performed in triplicate. Interestingly, the best results on % yield and total phenolics were exhibited by aqueous methanolic extract from UAE method. The mounting interest in plant derived phenolics is prompting the rapid research for fast, versatile, inexpensive, simple and green modern extraction technologies to overcome the technical limitations of conventional methods. Hence, UAE has appeared as a promising method to combat the requisite criteria of an ideal extraction.

Acknowledgements

The present research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The authors declare that they have no conflict of interests.

References

Abdel-Sattar E, Harraz FM, Al-Ansari SMA, El-Mekkawy S, Ichino C, Kiyohara H, Ishiyama **A**, Otoguro K, *et al*.,Yamada H (2008). Acylated pregnane glycosides from *Caralluma tuberculata* and their antiparasitic activity. Phytochemistry 69(11):2180-2186.

Abdel-Sattar E, Harraz FM, Ghareib SA, Elberry AA, Gabr S, Suliaman MI (2011). Antihyperglycaemic and hypolipidaemic effects of the methanolic extract of *Caralluma tuberculata* in streptozotocin-induced diabetic rats. Natural Product Research 25(12):1171-1179.

Adnan M, Jan S, Mussarat S, Tariq A. Tariq A, Begum S, *et al*., Shinwari ZK (2014). A review on ethnobotany, phytochemistry and pharmacology of plant genus *Caralluma* R. Br. Journal of Pharmacy and Pharmacology 66(10):1351-1368.

Ahmad K, Khan ZI, Ashraf M. Hussain M, Ibrahim M, Valeem EE (2008) Status of plant diversity at Kufri (Soone valley) Punjab, Pakistan and prevailing threats therein. Pakistan Journal of Botany 40(3):993-997.

Ahmad S (1995). Oxidative stress from environmental pollutants. Archives of Insect Biochemistry and Physiology 29(2):135-157.

Ahmad VU, Usmanghani K, Rizwani GH (1988). New pregnane glycosides from *Caralluma tuberculata*. Journal of Natural Products 51(6):1092-1097.

Ahmed M, Qureshi S, Al-Bekairi AM, Shah A.H, Rao RM, Qazi NS (1993). Anti-inflammatory activity of *Caralluma tuberculata* alcoholic extract. Fitoterapia 64(4):357-360.

Al-Faifi ZI, Zarraq AI, Masrahi YS, Aly MS (2016). *In vitro* anticancer activity of *Caralluma acutangula* (Decne.) NE Br. Extract. International Journal of Pharmaceutical Sciences Review and Research 38(2):59-63.

Ameer K, Shahbaz HM, Kwon JH (2017). Green extraction methods for polyphenols from plant matrices and their byproducts: A review. Comprehensive Reviews in Food Science and Food Safety 16(2):295-315.

Antolovich M, Prenzler P, Robards K, Ryan D (2000). Sample preparation in the determination of phenolic compounds in fruits. Analyst 125(5):989-1009.

Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Analytical Biochemistry 239(1):70-76.

Buttke TM, Sandstrom PA (1994). Oxidative stress as a mediator of apoptosis. Immunology Today 15(1):7-10.

Chen C (2015). Sinapic acid and its derivatives as medicine in oxidative stress-induced diseases and aging. Oxidative Medicine and Cellular Longevity.

Chen ZR. Bertin, Froldi G (2013). EC 50 estimation of antioxidant activity in DPPH assay using several statistical programs. Food Chemistry 138(1):414-420.

Clarke G, Ting KN, Wiart C, Fry J (2013). High correlation of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, ferric reducing activity potential and total phenolics content indicates redundancy in use of all three assays to screen for antioxidant activity of extracts of plants from the Malaysian rainforest. Antioxidants 2(1):1-10.

Deepak D, Khare A, Khare MP (1998). Plant pregnanes. Phytochemistry 28(12):3255-3263.

Dewanto V, Wu X, Adom KK, Liu RH (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50(10):3010-3014.

Dhanani T, Shah S, Gajbhiye NA, Kumar S (2017). Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. Arabian Journal of Chemistry 10: S1193-S1199.

Farouk AE, Ahamed NT, AlZahrani O, Alamer KH, Al-Sodany Y, Bahobail A (2016). Antimicrobial activity of *Caralluma quadrangula* (Forssk) NE Br latex from Al-Shafa Taif, Kingdom of Saudi Arabia. International Journal of Current Microbiology and Applied Science 5(11):284-298.

Furukawa S, Fujita T, Shimabukuro M. Iwaki M, Yamada Y, *et al*., Shimomura I (2017). Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of Clinical Investigation 114(12): 1752-1761.

Jyoti DN, Kishore M, Rao YH (2015). A medicinal importance and chemical composition of *Caralluma umbellata* Haw. International Journal of Advanced Research in Chemical Science (IJARCS) 2(3):33-37.

Khan SW, Khatoon S (2008). Ethnobotanical studies on some useful herbs of Haramosh and Bugrote valleys in Gilgit, northern areas of Pakistan. Pakistan Journal of Botany 40(1):43-58.

Khasawneh M, Elwy HM, Fawzi NM, Hamza AA, Chevidenkandy AR, Hassan AA (2014). Antioxidant activity and lipoxygenase inhibitory effect of *Caralluma arabica* and related polyphenolic constituents. American Journal of Plant Sciences 5(11):1623-1631.

Kommidi D, Koorbanally N, Moodley B (2016). Lung cancer activity of *Caralluma* species: an overview. Austin Journal of Lung Cancer

Research 1(2):1006.

Li F, Li Q, Gao D, Peng Y, Feng C (2009). Preparation and antidiabetic activity of polysaccharide from *Portulaca oleracea* L. African Journal of Biotechnology 8(4):569-573.

Liede-Schumann S, Rapini A, Goyder DJ, Chase MW (2005). Phylogenetics of the new world subtribes of Asclepiadeae (Apocynaceae-Asclepiadoideae): Metastelmatinae, oxypetalinae, and gonolobinae. Systematic Botany 30(1):184-195.

Lou Z, Wang H, Rao S, Sun J, Ma C, Li J (2012). p-Coumaric acid kills bacteria through dual damage mechanisms. Food Control 25(2):550-554.

Mahmood T, Muhammad S, Shinwari ZK (2010). Molecular and morphological characterization of *Caralluma* species. Pakistan Journal of Botany 42(2):1163-1171.

Mitra I, Saha A, Roy K (2010). Exploring quantitative structure-activity relationship studies of antioxidant phenolic compounds obtained from traditional Chinese medicinal plants. Molecular Simulation 36(13):1067-1079.

Ow YY, Stupans I (2003). Gallic acid and gallic acid derivatives: effects on drug metabolizing enzymes. Current Drug Metabolism 4(3):241-248.

Parr AJ, Bolwell GP (2000). Phenols in the plant and in man. The potential for possible nutritional enhancement of the diet by modifying the phenols content or profile. Journal of the Science of Food and Agriculture 80(7):985-1012.

Peschel W, Sánchez-Rabaneda F, Diekmann W, Plescher A, Gartzia I, Jimenez D, *et al*., Codina C (2006). An industrial approach in the search of natural antioxidants from vegetable and fruit wastes. Food Chemistry 97(1):137-150.

Proestos C, Lytoudi K, Mavromelanidou OK, Zoumpoulakis P, Sinanoglou VJ (2013). Antioxidant capacity of selected plant extracts and their essential oils. Antioxidants 2 (1):11-22.

Sultana B, Anwar F (2008). Flavonols (kaempeferol, quercetin, myricetin) contents of selected fruits, vegetables and medicinal plants. Food Chemistry 108(3):879-884.

Sultana B, Anwar F, Przybylski R (2007). Antioxidant activity of phenolic components present in barks of *Azadirachtaindica*, *Terminalia arjuna*, *Acacia nilotica*, and *Eugenia jambolana* Lam. Trees. Food Chemistry 104(3):1106-1114.

Thaipong K, Boonprakob U, Crosby K, Crosby K, Cisneros-Zevallos L, Byrne DH (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19(6):669-675.

Uttara B, Singh AV, Zamboni P, Mahajan R (2009). Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. Current Neuropharmacology 7(1):65-74.

Valko M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 160(1):1-40.

Zima T, Aacute Scaron, L Mestek O, Janebová M, *et al*., Popov P (2001). Oxidative stress, metabolism of ethanol and alcohol-related diseases. Journal of Biomedical Science 8(1):59-70.