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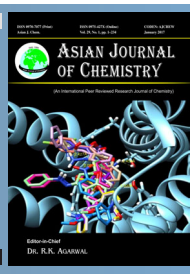

Effect of Extraction Techniques on Total Phenolics, Flavonoids Contents and Antioxidant Activity of Various Solvent Fractions of Bark of Arjun (*Terminalia arjuna*)

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The objective of the present work was to investigate the effect of extraction techniques *viz.* cold (mechanical shaking) and hot (Soxhlet) on total phenolics and flavonoids as well as on the antioxidative potential of methanolic extracts of *Terminalia arjuna* bark and its fractions in solvents of different polarities. Results revealed that extract yield (12.623 g/100 g), total phenolics content (45.377 mg GAE/g), flavonoids content (15.071 mg CE/g) and DPPH free radical scavenging activity (IC_{50}: 134.8 µg/mL) were found to be higher in methanolic extract obtained by hot extraction technique in comparison to cold extraction technique. In various solvent fractions of methanolic extracts of bark of Arjun obtained by both extraction techniques, total phenolics and flavonoids contents were found to be highest in residual aqueous fraction followed by butanol, ethyl acetate, dichloromethane, chloroform and hexane fractions. The methanolic extracts obtained by both extraction techniques, ethyl acetate fraction exhibited the highest antioxidant activity followed by butanol, residual aqueous, chloroform, dichloromethane and hexane fractions.

Keywords: *Terminalia arjuna*, Extraction techniques, Solvent fractions, Phenolics, Flavonoids, DPPH free radical scavenging activity.

INTRODUCTION

Human body naturally produces free radicals and the antioxidants to counteract their damaging effects. However, capacity of the defensive system is affected by age, diet, health status of individual [1]. To keep proper equilibrium between ROS and defense system components, there is a need to provide antioxidants as part of diet [2]. Antioxidants also play important role in preventing oxidative deterioration of food and indirectly eliminating radicals from it [3]. In general, there are two basic categories of antioxidants, natural and synthetic. Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity [4]. It has also been proposed that antioxidant activity of plant origin components can be mainly ascribed to the presence of phenolic compounds [5]. It is generally known that the yield of chemical extraction depends on the type of solvents with varying polarities, extraction time and temperature, sample-to-solvent ratio as well as on the chemical composition and physical characteristics of the samples. Solvents such as methanol, ethanol, acetone, ethyl acetate and their combinations have been used for the extraction of phenolics from plant materials, often with different proportions of water. Selecting the right solvent affects the amount and rate of polyphenols extracted [6].

The plant *Terminalia arjuna* L. commonly known as Arjun, a remarkable tree for its important phytochemicals belongs to family combretaceae or the terminalia [7]. Arjun is found throughout the South Asian region. This tree is usually evergreen tree with new leaves appearing in the season February to April before leaf fall. This tree is an exotic tree in India. Various parts of Arjun are good source of phytosterol, namely, β-sitosterol which lowers down the cholesterol in blood serum mediated through inhibition of cholesterol absorption resulting from the higher solubility of phytosterol than of cholesterol in bile salt micelles [8,9]. Flavonoids present in *T. arjuna* bark have been reported to exert antioxidant, anti-inflammatory and lipid lowering effects while glycosides are cardiotonic, thus making *T. arjuna* unique amongst most commonly used medicinal plant in Indian subcontinent [10]. Survey of literature reveals that less work has been done on the effect of cold and hot extraction techniques on phytochemicals and antioxidant activity of different solvent fractions of bark of Arjun. Thus, objective of present study was to study the effect of extraction techniques towards extraction of total phenolics and flavonoids content as well as on antioxidant activity of various solvent fractions of bark extracts of Arjun (*Terminalia arjuna*).

EXPERIMENTAL

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merck and Hi-Media of highest purity, were used for various experimental procedures.

Plant material and extraction: Bark sample of Arjun (*Terminalia arjuna*) was procured from the experimental area of Medicinal, Aromatic and Potential Crops Section, Department of Genetics and Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University, Hisar, India. Arjun bark was dried and ground in grinding machine to obtain a fine powder. Powdered samples were extracted by using following two extraction techniques:

Cold (mechanical shaking): 25 g of powdered samples of bark of Arjun were extracted with 175 mL of methanol in 500 mL conical flasks by shaking on a mechanical shaker for 2 h. In each set, total eight conical flasks were placed on mechanical shaker. Extracts were filtered and residues were again extracted twice (shaking time 1 h) with 125 and 100 mL methanol taken in each conical flask. Filtrates from three extraction steps were pooled and their volumes were noted. Several steps of mechanical shaking were repeated to have sufficient amount of extract required for various studies.

Hot (Soxhlet extraction): 200 g of powdered samples of bark of Arjun were placed in a filter paper (Whatman No. 1) thimble in a classical Soxhlet apparatus fitted with a 3 L round bottom flask. The methanol was added up to one and a half siphons that is approximately 1400 mL. Extraction was performed at boiling temperature of methanol, solvent vapours move up to the column and after getting condensed in the condenser part, floods into the chamber housing thimble filled with Arjun bark samples. When this chamber was filled completely with solvent, the siphon mechanism operates and the solvent containing some part of phytochemicals that got dissolved in solvent; empties this extract into round bottom flask containing solvent. Process was continued for 14 h with completion of up to seven to eight cycles through siphon mechanism. After the completion of extraction step, residue in thimble was again extracted twice (each extraction time 10 and 8 h, respectively) with suitable amount of methanol. Filtrates from three extraction steps were pooled and their volumes were noted. Several steps of Soxhlet extraction were repeated to have sufficient amount of extract required for various studies.

All the samples extracted by using above mentioned techniques were performed in triplicate. All extracts were bottled properly and stored in refrigerator at 4 °C.

Liquid-liquid partitioning/extraction: 500 mL of each extract obtained by using cold or hot extraction technique was concentrated on rotary vacuum evaporator under reduced pressure followed by manifold evaporator till viscous mass was obtained. Viscous mass was defatted by washing 3-4 times with petroleum ether (60-80 °C). Defatted viscous mass was dissolved in 100 mL of 10 % methanol in distilled water, precipitation occurred and it was allowed to separate into aqueous layer and non-aqueous layer (precipitates). Both of these layers were separated by filtration. Further the aqueous layer was partitioned into various solvent fractions. Liquid-liquid partitioning/extraction was achieved by shaking the aqueous layer and solvent in a separating funnel *i.e.* successively partitioned with hexane (30, 20, 20 mL), dichloromethane (30, 20, 20 mL), chloroform (30, 20, 20 mL), ethyl acetate (60, 40, 40 mL) and butanol (40, 30, 30 mL) in sequence. However, occasionally large amount of emulsions were formed (except in partitioning with butanol) and it was difficult to separate out the solvent from the aqueous layer even after keeping it long time, then emulsion was broken down by adding a small amount (2-3 mL) of ethanol. All the volumes of each solvent fraction from three partitioning steps were pooled and their volumes were noted. Non-aqueous layer (precipitates) was redissolved in suitable amount of methanol (30-60 mL) and volume was noted.

Methanolic extracts of bark of Arjun and its various solvent fractions were used for estimation of total phenolics and flavonoids content and for evaluation of antioxidant activity by DPPH free radical scavenging activity method.

Estimation of total phenolics: Total phenolics content of extracts was determined using Folin-Ciocalteu method [11]. Aliquots of 0.2 mL of extracts were mixed with 1 mL of 1 mol/L Folin-Ciocalteu reagent. After that, 2.0 mL of 20 % (w/v) sodium carbonate solution was added. The solutions were mixed and volume was made up to 10 mL with distilled water. The absorbance was measured at 730 nm using UV-visible double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using gallic acid as standard. Results were expressed as mg GAE/g on dry weight basis.

Estimation of total flavonoids: Flavonoids content of extracts was estimated according to the colorimetric assay [12]. In 1 mL of extract, 4 mL of double distilled water and 0.3 mL of 5 % (w/v) $NaNO_2$ were added. After 5 min, 0.3 mL of 10 % (w/v) $AlCl_3$ was added. Immediately, 2 mL of 1 M NaOH was added and the volume was made up to 10 mL with double distilled water. The solution was mixed thoroughly and the absorbance was measured at 510 nm using UV-visible double beam Spectrophotometer Model 2203 (Systronics Co.). A calibration curve was prepared using catechin as standard. Results were expressed as mg CE/g on dry weight basis.

DPPH free radical scavenging activity: The antioxidant activity of the extracts was evaluated by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method [13]. Extracts were dried up completely and the weight of dry mass was noted. The dry mass of methanolic extract and various solvent fractions *viz.* hexane, dichloromethane, chloroform, ethyl acetate and butanol were redissolved in appropriate amount of methanol to make the stock solution (5000 µg/mL). Since, the dry mass of water extract (residual aqueous fraction) was not soluble in pure methanol, hence, it was redissolved in 50 % (v/v) methanol : water to make the stock solution. From stock solution, different concentrations (25 µg/mL to 5000 µg/mL) were made by appropriate dilutions with methanol for various solvent fractions and 50 % (v/v) methanol:water for residual aqueous fraction. For evaluation of antioxidant activity, in 0.2 mL extract of each concentration, 3 mL of 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.1 mM in 100 % methanol) was added and mixed thoroughly for 5 min. For

antioxidant activity in residual aqueous fraction, DPPH stock solution was prepared in 50 % (v/v) methanol:water and remaining procedure was same. A control was also made containing 0.2 mL of methanol instead of extract. The absorbance of the sample as well as control was measured at 517 nm after 30 min of incubation in dark at room temperature using the UV-visible double beam Spectrophotometer Model 2203 (Systronics Co.) against a blank containing methanol. A graph was drawn by plotting percent DPPH free radical scavenging activity (y-axis) against extract concentration (x-axis). Then using the Microsoft Excel Software, a quadratic regression equation ($y = ax^2 + bx + c$) was obtained. By putting $y = 50$ (for IC_{50}) in the equation $y = ax^2 + bx + c$; it was converted to the form $ax^2 + bx + c = 0$. IC_{50} was calculated from the equation $ax^2 + bx + c = 0$ by using the formula:

$$x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where, $x = IC_{50}$ (µg/mL).

Calculation: The percentage of DPPH scavenged (% $DPPH^*_{sc}$) was calculated using:

$$DPPH^*_{sc}\ (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ is the absorbance of control and A_{sample} is the absorbance of the sample.

RESULTS AND DISCUSSION

Extract yield: Extract yield of methanolic extracts of bark of Arjun and its aqueous and non-aqueous layers is given in Table-1. Methanolic extract of bark of Arjun obtained by using hot extraction technique had higher extract yield *i.e.* 12.623 g/100 g in comparison to cold extraction technique *i.e.* 6.528 g/100 g. Aqueous and non-aqueous layers of methanolic extract obtained by hot extraction technique also had higher extract yield *i.e.* 8.255 and 4.368 g/100 g, respectively in comparison to cold extraction technique *i.e.* 3.034 and 3.493 g/100 g, respectively. Our findings are in agreement with previous investigation which reported that burdock datura (*Xanthium strumarium*) extract obtained by Soxhlet method has higher extract yield in comparison to extracts obtained by static maceration and dynamic maceration (shaking) methods and this higher extract yield of extract obtained by Soxhlet method is due to higher temperature, which may have increased the strength of solvation [14].

Aqueous layers of methanolic extracts of bark of Arjun were partitioned into various solvent fractions. The data of extract yield (g/100 g) of various solvent fractions of aqueous layers of methanolic extracts of bark of Arjun obtained by both extraction techniques is given in Table-2. Extract yield of various solvent fractions of methanolic extract obtained by cold extraction technique varied from 0.008 to 2.523 g/100 g and decreased in the following order: residual aqueous > butanol > ethyl acetate > chloroform > hexane > dichloromethane. In case of extract yield of various solvent fractions of methanolic extract obtained by hot extraction technique, it varied from 0.014 to 6.497 g/100 g and decreased in the following order: residual aqueous > butanol > ethyl acetate > dichloromethane > chloroform > hexane. Among various solvent fractions of wild parsley (*Torilis leptophylla*), residual aqueous fraction has highest extract yield *i.e.* 8.2 g/100 g followed by ethyl acetate (6.1 g/100 g), butanol (4.8 g/100 g) and chloroform (4.3 g/100 g) fractions [15]. Amongst different solvent extracts of bark of *T. arjuna*, chloroform had higher extract yield (g/100 g) *i.e.* 0.80 in comparison to hexane (0.35) extract [16].

Total phenolics content: Total phenolics content of methanolic extracts of bark of Arjun and its aqueous and non-aqueous layers is given in Table-1. Methanolic extracts of bark of Arjun obtained by hot extraction technique contained higher total phenolics content *i.e.* 45.377 mg GAE/g in comparison to cold extraction technique *i.e.* 13.251 mg GAE/g. Aqueous and non-aqueous layers of methanolic extract obtained by hot extraction technique also contained higher total phenolics

TABLE-1
EXTRACT YIELD, TOTAL PHENOLICS AND FLAVONOIDS IN METHANOLIC EXTRACTS OF BARK OF ARJUN AND IN ITS AQUEOUS AND NON-AQUEOUS LAYERS

Extract/layers	Cold extraction technique			Hot extraction technique		
	Extract yield (g/100 g)	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	Extract yield (g/100 g)	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)
Methanol	6.528 ± 0.028	13.251 ± 0.255	4.353 ± 0.064	12.623 ± 0.132	45.377 ± 0.184	15.071 ± 0.134
Aqueous	3.034 ± 0.072	6.766 ± 0.038	1.972 ± 0.057	8.255 ± 0.067	17.910 ± 0.063	5.988 ± 0.092
Non-aqueous	3.493 ± 0.040	6.485 ± 0.035	2.381 ± 0.026	4.368 ± 0.023	27.467 ± 0.115	9.083 ± 0.088

TABLE-2
EXTRACT YIELD, TOTAL PHENOLICS AND FLAVONOIDS IN VARIOUS SOLVENT FRACTIONS OF METHANOLIC EXTRACTS OF BARK OF ARJUN OBTAINED BY HOT AND COLD EXTRACTION TECHNIQUES

Fractions	Cold extraction technique			Hot extraction technique		
	Extract yield (g/100 g)	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)	Extract yield (g/100 g)	Total phenolics (mg GAE/g)	Total flavonoids (mg CE/g)
Hexane	0.011 ± 0.001	0.010 ± 0.001	0.004 ± 0.000	0.014 ± 0.002	0.011 ± 0.000	0.010 ± 0.000
Dichloromethane	0.008 ± 0.001	0.052 ± 0.001	0.010 ± 0.000	0.024 ± 0.002	0.082 ± 0.002	0.021 ± 0.001
Chloroform	0.014 ± 0.001	0.045 ± 0.001	0.005 ± 0.001	0.017 ± 0.001	0.070 ± 0.001	0.016 ± 0.000
Ethyl acetate	0.144 ± 0.003	1.376 ± 0.004	0.387 ± 0.006	0.427 ± 0.008	3.165 ± 0.019	0.906 ± 0.006
Butanol	0.334 ± 0.001	1.680 ± 0.013	0.469 ± 0.013	1.276 ± 0.018	5.351 ± 0.033	1.585 ± 0.006
Residual aqueous	2.523 ± 0.025	3.603 ± 0.021	1.097 ± 0.027	6.497 ± 0.034	9.231 ± 0.027	3.450 ± 0.008

content *i.e.* 17.910 and 27.467 mg GAE/g, respectively in comparison to cold extraction technique *i.e.* 6.766 and 6.485 mg GAE/g, respectively. The findings are in agreement with the previous study which reported that clove buds extracts obtained by using Soxhlet extraction technique had higher total phenolics in comparison to extracts obtained by mechanical shaking extraction technique and this is due to the increased solubility of phenols in the extracting solvent had higher extraction temperature [17].

Aqueous layers of methanolic extracts of bark of Arjun were partitioned into various solvent fractions. Total phenolics content (mg GAE/g) of various solvent fractions of aqueous layers of methanolic extracts of bark of Arjun obtained by both extraction techniques is given in Table-2. Total phenolics content of various solvent fractions of methanolic extract obtained by cold extraction technique varied from 0.010 to 3.603 mg GAE/g and decreased in the following order: residual aqueous > butanol > ethyl acetate > dichloromethane > chloroform > hexane. In case of total phenolics content of various solvent fractions of methanolic extract obtained by hot extraction technique, it varied from 0.011 to 9.231 mg GAE/g and decreased in the following order: residual aqueous > butanol > ethyl acetate > dichloromethane > chloroform > hexane. Total phenolics present in bark of Arjun are mainly gallic acid, ellagic acid, Arjunin, Arjunone and Arjunolone which are polar in nature. The probable reason for higher amount of total phenolics in polar solvents may be due to presence of more polar phenolic compounds. Ethyl acetate fraction of garden mint (*Mentha spicata)* had highest total phenolics content (mg GAE/g) *i.e.* 54 followed by chloroform (30) and hexane (14) fractions. Among different solvent fractions of *Cleome gynandra*, the total phenolics (mg GAE/g of fraction) content was highest in butanol (133.02) fraction followed by ethyl acetate (97.90), dichloromethane (37.380) and hexane (20.72) fractions and similarly, in different solvent fractions of *Maerua angolensis*, the total phenolics (mg GAE/g of fraction) content was highest in butanol (107.55) fraction followed by ethyl acetate (69.51), dichloromethane (40.81) and hexane (13.43) fractions [18]. It was also reported by some research workers that total phenolics content in water, ethyl acetate and hexane extracts of *T. arjuna* bark obtained by using Soxhlet apparatus was 3.6, 4.1 and 0.0 mg GAE/g, respectively [19].

Total flavonoids content: Total flavonoids content of methanolic extracts of bark of Arjun and its aqueous and non-aqueous layers is given in Table-1. Methanolic extract of bark of Arjun obtained by hot extraction technique contained higher total flavonoids content *i.e.* 15.071 mg CE/g in comparison to cold extraction technique *i.e.* 4.353 mg CE/g. Aqueous and non-aqueous layers of methanolic extract obtained by hot extraction technique also contained higher total flavonoids content *i.e.* 5.988 and 9.083 mg CE/g, respectively in comparison to cold extraction technique *i.e.* 1.972 and 2.381 mg CE/g, respectively. A review of previously documented literature also revealed that clove buds extracts obtained by using Soxhlet extraction technique also had higher total flavonoids in comparison to extracts obtained by mechanical shaking extraction technique [17].

Aqueous layers of methanolic extracts of bark of Arjun were partitioned into various solvent fractions. Total flavonoids content (mg CE/g) of various solvent fractions of aqueous layers of methanolic extracts of bark of Arjun obtained by both extraction techniques is given in Table-2. Total flavonoids content of various solvent fractions of methanolic extract obtained by cold extraction technique varied from 0.004 to 1.097 mg CE/g and decreased in the following order: residual aqueous > butanol > ethyl acetate > dichloromethane > chloroform > hexane. In case of total flavonoids content of various solvent fractions of methanolic extract obtained by hot extraction technique, it varied from 0.010 to 3.450 mg CE/g and decreased in the following order: residual aqueous > butanol > ethyl acetate > dichloromethane > chloroform > hexane. Flavonoids present in bark of Arjun are mainly bicalein, quercetin, kaemperol, luteolin, pelorgonidin which are polar compounds. The probable reason for higher amounts of total flavonoids in polar solvents may be due to presence of more polar flavonoid compounds. Total flavonoids (mg CE/g) content in different solvent fractions of chir pine (*Pinus roxburgii)* was highest in ethyl acetate (428) fraction followed by butanol (391), dichloromethane (160) and hexane (108) fractions [19]. Total flavonoids content (mg QE/g) was also much higher in water (6.1) and ethyl acetate (9.1) extracts than hexane (0.0) extracts of *T. arjuna* bark [20].

DPPH free radical scavenging activity: 2,2'-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical (purple colour) and it transforms to non radical form (yellow colour) by abstracting one electron and hence, it is widely used as a measure for the electron donation capacity of antioxidants under assay conditions [21]. DPPH free radical scavenging activity of methanolic extracts of bark of Arjun obtained by cold and hot extraction techniques ranged from 5.96 to 95.83 % and from 10.50 to 95.67 %, respectively at 25 to 5000 µg/mL concentration levels. IC_{50} values (Fig. 1) were calculated from the quadratic regression equations (Table-5). IC_{50} value of methanolic extract of bark of Arjun obtained by hot extraction technique was lower *i.e.* 134.8 µg/mL in comparison to cold extraction technique (180.5 µg/mL) thereby showing that methanolic extract obtained by hot extraction technique exhibited higher activity in comparison to cold extraction technique. The present findings are in agreement with the studies on clove buds which reported that extracts obtained by Soxhlet technique possessed the highest antioxidant activity followed by refluxing, mechanical shaking and centrifugation due to the presence of higher amount of antioxidants compounds *i.e.* total phenolics and flavonoids in the extracts obtained by Soxhlet technique [17]. It has also been reported that methanol extract of bark of *T. arjuna* showed maximum activity of 94.72 % at 250 µg/mL [15].

DPPH free radical scavenging activity (%) of various solvent fractions of methanolic extracts of bark of Arjun obtained by both extraction techniques was concentration dependent. It increased with increase in concentration levels and the data is given in Tables 3 and 4. Amongst various solvent fractions of methanolic extract of bark of Arjun obtained by cold extraction technique, ethyl acetate fraction has the lowest IC_{50} value *i.e.* 38.1 µg/mL followed by butanol (76.2 µg/mL), residual aqueous

TABLE-3
DPPH FREE RADICAL SCAVENGING ACTIVITY (%) OF METHANOLIC EXTRACT OF BARK OF ARJUN OBTAINED BY USING COLD EXTRACTION TECHNIQUE AND OF ITS VARIOUS SOLVENT FRACTIONS

Conc. (µg/mL)	DPPH free radical scavenging activity (%): Extract/Fractions						
	Methanol	Hexane	Dichloromethane	Chloroform	Ethyl acetate	Butanol	Residual aqueous
5000	95.83	–*	89.89	86.10	94.57	92.70	85.39
2500	94.97	26.11	88.99	85.25	94.15	91.95	84.78
1000	94.43	10.86	77.17	79.69	94.02	91.78	84.40
500	93.15	5.53	44.02	53.36	93.31	91.10	80.89
250	66.34	3.60	23.80	26.74	93.17	90.13	48.47
100	30.80	1.97	12.52	12.89	92.59	57.30	21.25
50	11.49	1.77	5.03	2.77	66.28	34.30	14.98
25	5.96	0.98	2.06	0.34	32.27	14.72	7.65

*Dry mass of hexane fraction was not sufficient to prepare solution of 5000 µg/mL.

TABLE-4
DPPH FREE RADICAL SCAVENGING ACTIVITY (%) OF METHANOLIC EXTRACT OF ARJUN BARK OBTAINED BY USING HOT EXTRACTION TECHNIQUE AND OF VARIOUS SOLVENT FRACTIONS

Conc. (µg/mL)	DPPH free radical scavenging activity (%): Extract/Fractions						
	Methanol	Hexane	Dichloromethane	Chloroform	Ethyl acetate	Butanol	Residual aqueous
5000	95.67	–*	89.99	89.96	93.29	92.91	85.08
2500	94.98	32.39	89.36	89.56	92.74	91.92	84.78
1000	94.57	13.61	80.85	84.14	92.61	91.85	83.85
500	93.39	8.91	51.98	57.43	92.61	91.09	80.77
250	79.38	6.06	27.20	31.33	92.61	90.08	74.62
100	35.77	4.06	9.12	14.26	91.88	63.41	32.77
50	22.95	3.15	4.71	4.21	71.01	37.98	20.77
25	10.50	2.42	1.67	0.80	44.35	21.24	6.62

*Dry mass of hexane fraction was not sufficient to prepare solution of 5000 µg/mL.

TABLE-5
QUADRATIC REGRESSION EQUATIONS FOR DPPH FREE RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACTS OF BARK OF ARJUN OBTAINED BY USING COLD AND HOT EXTRACTION TECHNIQUES AND ITS VARIOUS SOLVENT FRACTIONS

Extract/Fractions	Quadratic regression equations	
	Cold extraction	Hot extraction
Methanol	$y = -0.004\times10^{-1}x^2 + 0.372x - 4.120$; $R^2 = 0.999$	$y = -0.005\times10^{-1}x^2 + 0.438x + 0.040$; $R^2 = 0.997$
Hexane	$y = 0.002\times10^{-4}x^2 + 0.010x + 1.017$; $R^2 = 0.999$	$y = 0.003\times10^{-4}x^2 + 0.011x + 2.749$; $R^2 = 0.998$
Dichloromethane	$y = -0.003\times10^{-2}x^2 + 0.103x + 0.256$; $R^2 = 0.999$	$y = -0.003\times10^{-2}x^2 + 0.115x - 0.730$; $R^2 = 0.998$
Chloroform	$y = -0.003\times10^{-2}x^2 + 0.116x - 0.680$; $R^2 = 0.995$	$y = -0.003\times10^{-2}x^2 + 0.122x + 0.595$; $R^2 = 0.993$
Ethyl acetate	$y = -0.003x^2 + 1.241x + 7.102$; $R^2 = 0.976$	$y = -0.002x^2 + 0.977x + 24.48$; $R^2 = 0.977$
Butanol	$y = -0.001x^2 + 0.758x - 1.951$; $R^2 = 0.997$	$y = -0.001x^2 + 0.775x + 3.133$; $R^2 = 0.999$
Residual aqueous	$y = -0.001\times10^{-1}x^2 + 0.228x + 1.870$; $R^2 = 0.998$	$y = -0.006\times10^{-1}x^2 + 0.447x - 3.294$; $R^2 = 0.995$

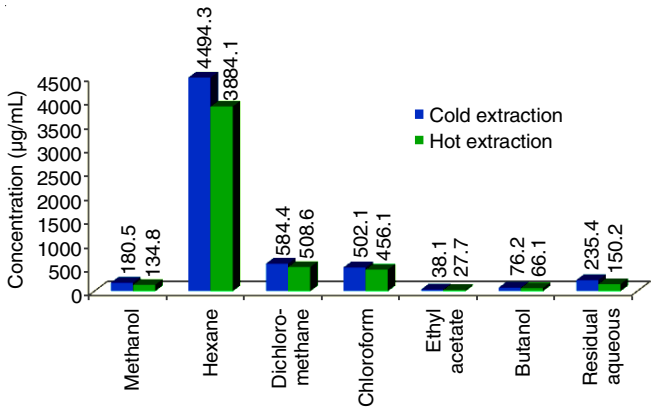

Fig. 1. IC_{50} values of methanolic extracts of bark of Arjun obtained by using cold and hot extraction techniques and various solvent fractions

(235.4 µg/mL), chloroform (502.1 µg/mL), dichloromethane (581.4 µg/mL) and hexane (4494.3 µg/mL) fractions thereby showing that ethyl acetate fraction exhibited the highest activity followed by butanol, residual aqueous, chloroform, dichloromethane and hexane fractions (Fig. 1). Similarly, in hot extraction technique the ethyl acetate fraction has the lowest IC_{50} value *i.e.* 27.7 µg/mL followed by butanol (66.1 µg/mL), residual aqueous (150.2 µg/mL), chloroform (456.1 µg/mL), dichloromethane (508.6 µg/mL) and hexane (3884.1 µg/mL) fractions thereby showing that ethyl acetate fraction exhibited the highest activity followed by butanol, residual aqueous, chloroform, dichloromethane and hexane fractions (Fig. 1). Other research workers also studied the effect of temperature and extraction process on the antioxidant activity of various organic crude extracts from the leaves of garden thyme (*Thymus vulgaris*) and found that amongst extracts prepared by Soxhlet extraction, the antioxidant activity was highest in butanol extract followed by methanol, ethyl acetate, chloroform and hexane extracts whereas amongst extracts prepared by maceration method, the activity was highest in ethyl acetate extract followed by methanol, butanol, hexane and chloroform extracts and this

trend may be due to polar organic solvents being more effective towards recovering optimal amount of antioxidant components from *T. vulgaris* [22]. In present study, the higher DPPH free radical scavenging activity was showed by various solvent fractions of aqueous layer of methanolic extract obtained by hot extraction technique (IC_{50} values 27.7 to 3884.1 µg/mL) in comparison to cold extraction technique (IC_{50} values 38.1 to 4494.3 µg/mL). Polar solvents *viz.* ethyl acetate, butanol, residual aqueous exhibited higher DPPH free radical scavenging activity in comparison to non-polar solvents *viz.* chloroform, dichloromethane and hexane. Hence, our results are in agreement with other research workers.

Conclusion

Bark of Arjun was found to be rich in total phenolics, flavonoids and exhibited good DPPH free radical scavenging activity. Amongst extraction techniques, methanolic extract of bark of Arjun obtained by hot extraction technique and its various solvent fractions contained higher total phenolics and flavonoids contents and also exhibited better DPPH free radical scavenging activity in comparison to cold extraction technique.

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