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Research article

Effects of extraction methods and solvent systems on extract yield, proximate composition and mineral profiling of *Terminalia arjuna* (Arjuna) dry powders and solvent extracts

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Abstract

Present experiment was conducted to evaluate and establish the effects of processing/pre-treatment on biochemical composition, mineral profiling and yield (%) of selected solvent extract of *Terminalia arjuna* (Arjuna). Analyses revealed that maximum yield (%) was ascertained in ethanolic bark extract for both fractions, individual (23.6±0.026%), and serial (22.23±0.017%). Non-significant difference ($p \geq 0.05$) was observed in yield of ethanolic and methanolic bark extracts while non-polar solvent extracts showed significant differences ($p \leq 0.05$). The mineral profiling revealed a wide variation among dry powder and their solvent extracts. After fractionation, the Zn content of fruit extracts increased and recorded highest to be in methanolic extract in the tune of 45.29 mg/l. The ash and moisture content established an inverse relationship for all solvent extracts. The maximum ash content was observed in arjuna bark powder 28.95±0.001% (serial) and 28.19±0.008 % (individual). The ash content does not follow the same pattern for mineral profiling ascribing more acid insoluble ash in bark followed by leaf and fruit which might be contributing towards bio-efficacy of the solvent extracts which can be depicted from present study that arjuna bark can be incorporated as ingredient for harnessing its bioactive properties and solvent extracts might be utilized for designing the drug. Thus, the present experiment showed a way to maximize the mineral profiling particularly Zn a potent neuro-transmitter which can be incorporated in developing the suitable feed for livestock's and fisheries in one hand, and pave a way for mitigating the nervous disorders in human health is concerned.

Abbreviations
L4: Acetone extract of arjuna leaf, L5: Ethanol extract of arjuna leaf, L6: Methanol extract of arjuna leaf, L7: Distilled water extract of arjuna leaf, Br4: Acetone extract of arjuna bark, Br5: Ethanol extract of arjuna bark, Br6: Methanol extract of arjuna bark, Br7: Distilled water extract of arjuna bark, F4: Acetone extract of arjuna fruit, F5: Ethanol extract of arjuna fruit, F6: Methanol extract of arjuna fruit, F7: Distilled water extract of arjuna fruit, AB: Arjuna Bark powder, AF: Arjuna Fruit powder, AL: Arjuna leaf powder.

Introduction

The medicinal plants have been used for multifarious purposes starting from timber as fuel to drug development [1-2]. The drug development properties of plants mainly depend on their secondary metabolite which provides the protection to the plants under adverse environmental conditions [3]. The secondary metabolite mainly includes the bioactive compounds which are very much integral component of the living system including human and

their associated organisms including fish. Bioactive compounds include the antioxidants, flavonoids, DNA damage protective compounds etc. [4]. DNA damage prevention activity indicates potential of herbal material to nullify the free radicals thereby providing the health befits and protection against de-oxygenerative diseases [5]. Crude extract of the herbal material are the witness of many untold story of the phytochemical and bioactive compounds such as anti-oxidant, flavonoids, tannin, saponin etc. present in it [6]. Since, long time anti-oxidant are being used in feed and beverage industries for extending the shelf life of the house hold material [7]. In addition, shelf-life of the feed of livestock and aqua-feed being enhanced using synthetic anti-oxidants which might be highly efficacious but relatively expensive and immunosuppressive and exert many side effects [8-9]. Gradually, the need of questing the alternate to these compounds is emerging on great pace as far as human health and ecosystem are concerned. The incorporation of such bioactive compounds in feed or other application involves a series of pre-treatment and processing for better palatability and flavors which may alter their pharmacological and ethno-medicinal properties [10]. The use of medicinal plants are increasing gradually and becoming popular remedies for common disease in day to day life [11]. The properties of medicinal plants depend on the spectrum of phytochemicals and other bioactive compounds. For instances, some plants are being recognized for their excellent ethno-medicinal properties and some are still not known till date [12]. In the era of tremendous market of medicinal plants, the developed countries are using 25% of medicinal plants out of total prescribed medicine and developing countries like India and china use 80% plants for ethno-medicinal purposes [13]. India is encompassing a diverse and better herbarium profile of excellent medicinal plants such as tulsi, neem, giloy, *Terminalia arjuna* etc., which have been becoming an integral part of their life and a sign of ethnicity also [14]. *Terminalia arjuna* (*arjuna*) is known for its versatility in terms of having wide spectrum of medicinal properties i.e. bark of *T. arjuna* is having anti-dysenteric, antipyretic, astringent, cardiotonic, lithotriptic, anticoagulant, hyperlipidemia, antimicrobial [15] and antiuremic properties [16]. The previous bibliographic studies on phytochemical profile of arjuna showed therapeutic and nutraceutically important compounds. Arjuna has been used in human diseases mediated through chronic degenerative actions, and showed strong antioxidant properties due to a number of important bioactive compounds. The application of Arjuna in prevention of human diseases involves either the decoction or extraction of a particular compound [17]. The success of therapeutic effects of medicinal plants depends extraction methods and solvent system and material to be extracted. Bibliographic studies showed that solvent extraction is good for anti-oxidant compounds. Orbital and magnetic shaking, refluxing and recently modern techniques are being used for compound isolation etc. however, the combination either of techniques could produce better result. This is a conventional approach which apparently used without seeing the availability of bioactive compound and their fractions. Further, traditional methods are not scientifically proven and the responsible compounds for efficacy are not known. The efficacy of plant/herbal material mainly depends on its organic compounds [18]. The information on proximate composition, mineral profiling of dry powder of different parts and their solvent extraction as individual fraction and serial fraction has not been done so far. In this back drop, the present experiment was executed to evaluate the effects of processing on Arjuna dry powder of three parts and their solvents extraction on crude and serially fractionated extracts on proximate composition, yield percentage and mineral profiling.

Material and methods

Collection of the material

There parts of Arjuna plant namely; bark, leaf and fruit were selected from the trees located in ICAR-CIFRI, Campus, Kolkata, India (Authentication no. 14) and confirmed for the botanical origin with the help of morphological characters and botanist.

Preparation of the extracts

After collection, the selected parts of Arjuna were washed in tap water to get rid of the extraneous material and dirt. After washing, the plant materials were subjected to sundry till removal of the moisture. Subsequently, the material were blended in the mixer and sieved through 50 micron sieve and the course materials again were put into the mixer till the course material appears not useful. About 100 g fine powder of each part was put into the individual four different solvent in 1:5 (material: solvent) *i.e.* acetone, methanol, ethanol and distilled water for individual fraction and for serial fraction, it was kept in hexane to remove the greasy materials and then kept in the four solvent serially starting from one polar aprotic (acetone), and three polar protic (methanol, ethanol and distilled water) for 36 hrs at 36 ^{o}C in a shaking incubator. After 36 hrs, the respective extracts were centrifuged in ReMi R-24(ReMi, India) for 5min at 8000rpm to settle down the course particle and then supernatant was collected in separate collection vial and then supernatant was subjected to filter through Whatman No.1 (40) filter paper and the remaining residue was once again kept for overnight in their respective solvents. The same procedure was repeated once and residue was discarded for individual fraction and for serial fraction the residue was allow to dry to evaporate the mother solvent and then kept in next polar solvent. The same procedure was

repeated for getting the supernatant. The supernatant was undergone the vacuum drying in rotary evaporator at below the boiling point of the solvent for proper distillation and kept till 1/10 of the original volume of the supernatant. The extracts then dried at room temperature and kept at 4 ^{o}C for further use.

Calculation of extract yield (%)

The yield (%) of solvent extracts was calculated for serial fractions and individual fractions as per the dry weight basis of the extracts with the following formula.

$$\text{Yield (\%)} = \frac{\text{Weight of the extract}}{\text{Weight of the total herbal material taken for extraction}} \text{ X } 100$$

Proximate composition

For evaluating the nutritional value, the proximate composition of the dry powder of the Arjuna extracts, individual and serial fraction were taken for analysis. The samples of selected fractions were dried at 105 ^{0}C to a constant weight to determine the moisture content. Crude protein was determined by measuring nitrogen (N % x 6.25) using micro Kjeldahl, crude lipid using Soxhlet apparatus and ash by combustion in a muffle furnace at 550 ^{0}C for 6 hrs [19].

Mineral profiling

The mineral profiling was done at ICAR-National Institute of Abiotic Stress Management, Malegaon-Karhavagaj Road, Khurd, Baramati, Maharashtra 413115, following AOAC [20]. Briefly, 1 g sample of each solvent extract was weighed and proceed for acidic digestion in microwave digestion system (Microwave Digestion System, Model START-D, SN- 135177, Milestone, USA). The HNO_3 and H_2O_2 were added in 5:1 ratio kept in digestion vessels for digestion [21]. After proper and complete digestion the samples were allowed to cool to room temperature then, digested samples were filtered with Whatman paper with 0.45 mm pore size and made up to 50 ml and proceed for trace elements analysis through Inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 series, Agilent Technologies, USA).

Statistical analysis

The statistical analysis was done using excel v.16, figures were edited in paint 3d v.16. The correlation matrix and box-violin plot with notches and outliers were established taking the relative value of individual fraction and serial fractions of solvent extracts in PAST 3.14 software. The values are represented as mean± standard error.

Results

Yield (%)

The yield (%) of both selected solvent extracts (individual and serial fraction) exhibits the narrow differences in yield (%) and varies from the nature of the parts used (Figure 1). Irrespective of solvents extracts the maximum yield % was recorded in the bark extracts for both the fractions, and the highest value was recorded for ethanolic extract of bark (23.6±0.026, 22.23±0.017%) followed by methanolic extract (22.7±0.016, 22.10±0.004%), acetone (11.36±0.005, 11.11±0.005%) and distilled water (4.1±0.048, 3.75±0.047%), for individual and serial fractions, respectively. The trend showing that bark extracts has maximum yield followed leaf and fruit extracts for polar protic solvents and pattern, and bark extract followed by fruit extract and leaf extracts was different in case of polar protic and polar aprotic, respectively (Table 1). The table 1 showed the comparative yield (%) of selected solvent extracts from Arjuna. The yield is a function of polarity and nature of solvents, and nature and physical structure of the herbal material to be extracted. Based on their response towards the solvent, they are assembling in clusters indicating the homogeneous groups (Figure 2). The bubble plot showing Br5 and Br6 are falling in same plain with avg. yield of more than 20%, followed by L4 and AF having yield of ≥10% and rest of the solvent extract encompassing yield ≤ 10%.

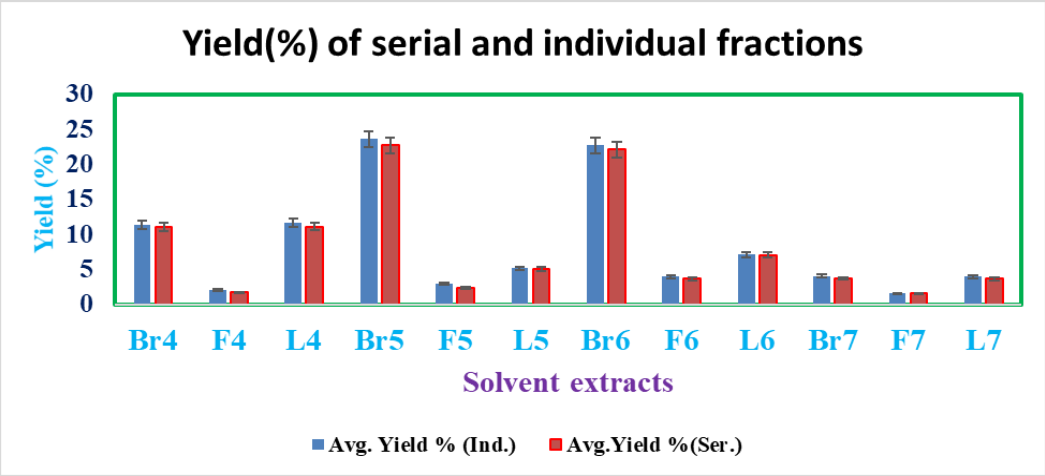

Figure 1. Comparative yield (%) of serial and individual fractions of Arjuna solvent extracts.

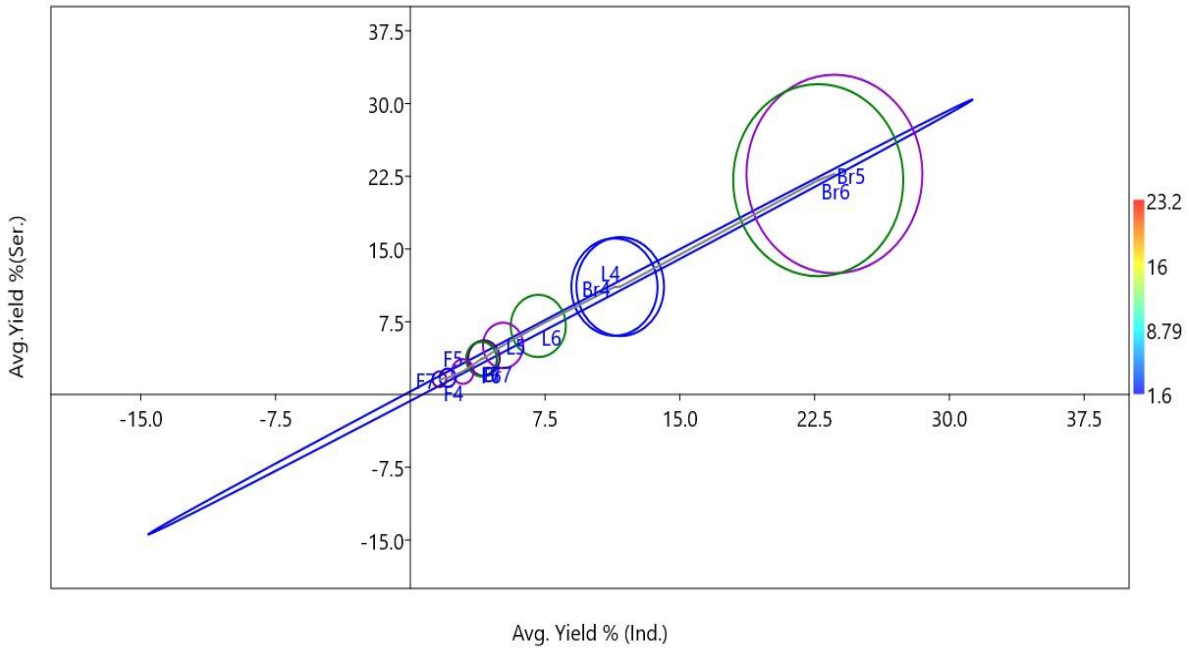

Figure 2. Bubble plot of Avg. yield (%) of serial fractions against Avg. yield (%) of individual fractions of Arjuna solvent extracts.

Table 1. Comparative yield (%) of serial and individual fractions of Arjuna solvent extracts.

Solvent Extracts	Avg. Yield (%) (Ind.)	Avg. Yield (%) (Ser.)
Br4	2.08±0.022	1.73±0.038
F4	11.68±0.031	11.12±0.002
L4	23.6±0.026	22.74±0.017
Br5	2.92±0.019	2.37±0.054
F5	5.16±0.033	5.067±0.006
L5	22.7±0.016	22.10±0.004
Br6	3.96±0.043	3.67±0.0072
F6	7.12±0.008	7.07±0.002
L6	4.1±0.048	3.75±0.047
Br7	1.62±0.052	1.57±0.009
F7	3.98±0.050	3.68±0.069
L7	2.08±0.022	1.73±0.038

Values are Mean± SE, N=6; here numerical 4, 5, 6 & 7 represent acetone, ethanol, methanol and distilled water and Br, F, and L presenting arjuna bark, fruit and leaf. The comparison was made between avg. yield of individual fraction and avg. yield of serial fraction.

Proximate composition

The maximum crude protein (%) content was recorded in Arjuna leaf powder (13.66±0.017) followed by fruit (5.29±0.044) and bark powder (3.53±0.067). The proximate composition of selected solvent extracts depicted the diverse state for proximate content (Table 2 & 3). The maximum variation was found in ash (%) of fractions which was recorded maximum in the leaf powder (11.48±0.020), followed by fruit (10.24±0.022) and bark (11.19±0.008) powder. While for individual and serial solvent fractions, it was recorded highest in methanolic extract of fruit (19.77±0.011; 19.53±0.001) followed by acetone (12.59±0.010; 13.35±0.001), distilled water (9.92±0.023; 9.68±0.002) and ethanolic extract of fruit (7.55±0.030; 7.31±0.002) for individual and serial fractions, respectively. The moisture content (%) followed inverse pattern with ash content as depicted in table 2 that before processing or extraction the minimum moisture (%) was reported in bark powder (6.36±0.030) followed by fruit (7.72±0.025) and leaf (10.2±0.019), respectively.

Table 2. Showing comparative proximate composition of Individual fractions of *T. arjuna* solvent extracts (% Dry matter basis).

Solvent extracts	CP (%)	CL (%)	Ash (%)	Moisture (%)
AF	5.29±0.044	1.82±0.088	10.24±0.022	7.72±0.025
AL	13.66±0.017	5.09±0.031	11.48±0.020	10.2±0.019
AB	3.53±0.067	1.07±0.150	28.19±0.008	6.36±0.030
Br4	1.77±0.133	1.26±0.127	10.57±0.010	5.36±0.035
F4	2.83±0.083	1.39±0.115	12.59±0.010	4.92±0.039
L4	11.47±0.020	1.54±0.104	11.46±0.020	9.84±0.019
Br5	1.66±0.142	1.44±0.111	1.95±0.116	9.66±0.020
F5	4.60±0.051	1.81±0.088	7.55±0.030	8.01±0.024
L5	12.56±0.019	3.68±0.043	11.36±0.020	9.93±0.019
Br6	2.11±0.112	1.79±0.089	7.57±0.030	8.08±0.024
F6	4.56±0.052	1.61±0.099	19.77±0.011	6.98±0.027
L6	12.50±0.019	2.5±0.064	9.46±0.024	10.15±0.019
Br7	2.42±0.097	1.03±0.155	9.55±0.024	9.84±0.019
F7	2.80±0.084	1.74±0.092	9.92±0.023	8.86±0.021
L7	4.82±0.049	1.52±0.105	7.62±0.030	7.87±0.024

Values are Mean± SE, N=6; here numerical 4, 5, 6 & 7 represent acetone, ethanol, methanol and distilled water and Br, F, and L presenting arjuna bark, fruit and leaf. The comparison was made between avg. yield of individual fraction and avg. yield of serial fraction. CP and CL representing crude protein and crude lipid content on dry matter basis.AF, AB and AL are being used for arjuna fruit, arjuna bark and arjuna leaf.

Table 3. Showing comparative proximate composition of serial fractions of *T. arjuna* solvent extracts.

Solvent extracts	CP (%)	CL (%)	Ash (%)	Moisture (%)
AF	5.53±0.086	1.99±0.164	10.00±0.002	7.92±0.049
AL	13.90±0.034	5.26±0.062	11.24±0.001	10.4±0.038
AB	3.77±0.126	1.24±0.263	28.95±0.001	6.56±0.059
Br4	2.01±0.237	1.43±0.228	11.33±0.001	5.56±0.070
F4	3.07±0.155	1.56±0.209	13.35±0.001	5.12±0.076
L4	11.71±0.041	1.71±0.191	12.22±0.001	10.04±0.039
Br5	1.90±0.251	1.61±0.202	1.71±0.009	9.86±0.040
F5	4.84±0.098	1.98±0.165	7.31±0.002	8.21±0.048
L5	12.80±0.037	3.85±0.085	11.12±0.001	10.13±0.038
Br6	2.35±0.203	1.96±0.166	7.33±0.002	8.28±0.047
F6	4.80±0.099	1.78±0.183	19.53±0.001	7.18±0.054
L6	12.74±0.037	2.67±0.122	9.22±0.002	10.35±0.038
Br7	2.66±0.179	1.20±0.272	9.31±0.002	10.04±0.039
F7	3.04±0.157	1.91±0.171	9.68±0.002	9.06±0.043
L7	5.06±0.094	1.69±0.193	7.38±0.002	8.07±0.048

Values are Mean± SE, N=6; here numerical 4, 5, 6 & 7 represent acetone, ethanol, methanol and distilled water and Br, F, and L presenting arjuna bark, fruit and leaf. The comparison was made between avg. yield of individual fraction and avg. yield of serial fraction. AF, AB and AL are being used for arjuna fruit, arjuna bark and arjuna leaf.

Mineral profiling

Mineral profiling of the selected solvent extracts for both types of extraction methods has completed for 14 important minerals (Table 4 & 5). The Arjuna fruit powder possesses the Zn in highest quantity (29.314±0.001mg/l) followed by bark powder (27.394±0.001mg/l) and leaf powder (23.16±0.002 mg/l), respectively. Following extraction by both methods resulted in lowering the minerals in ethanolic bark extracts as compared to the powder from and other bark solvent extracts. The highest Zn was recorded in methanolic fruit extracts (45.816±0.001mg/l and 47.021±0.001mg/l) for individual and serial fractions, respectively. Over all mineral profiling of powder forms of three parts revealed that fruit has better profiling followed by bark and leaf (Figure 4). While after extraction, the pattern has changed and the overall mineral profiling of powder forms and solvent extracts can be represented in ascending order as follows: F6>AF≥Br6>AB>L5≥Br4>AL>Br5>F5≥L7>L4>F7>Br7 >L6 (Figure 3). After taking the both fraction and crude powder forms in to consideration the correlation matrix showed an association at 5% level of significance. In Fig. 4, the empty block/cell showing insignificant differences while blue colored box/cells are showing significant difference at 5 %level of significance. Starting from Br4, it has significant (p<0.05) association with L4, L5, Br6, F6, L6, F7 and L7. Br5 has significant association

(p<0.05) with L4, L5, Br6, F6, L6, F6, Br7 and L7. Br6 has significant association with Br4, L4, Br5, L5, L6, F7 and L7.

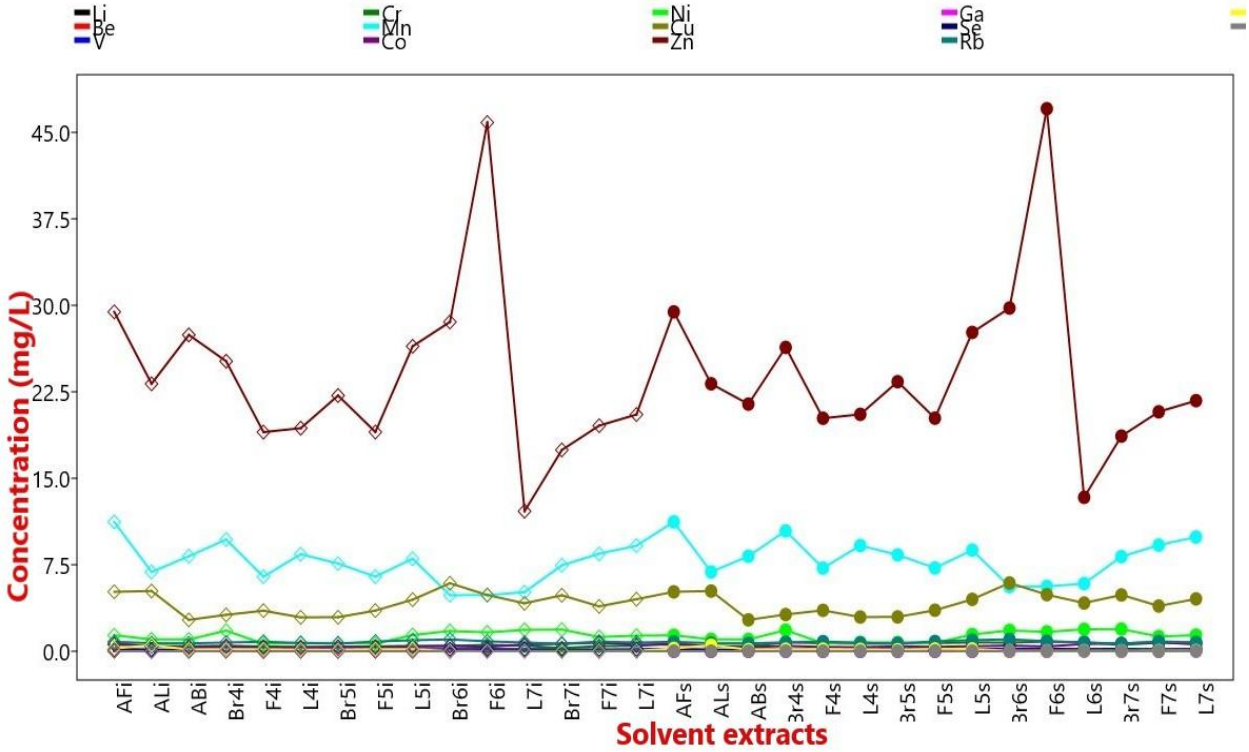

Figure 3. Showing comparative mineral profiling of individual and serial fractions of *T. arjuna* solvent extracts.

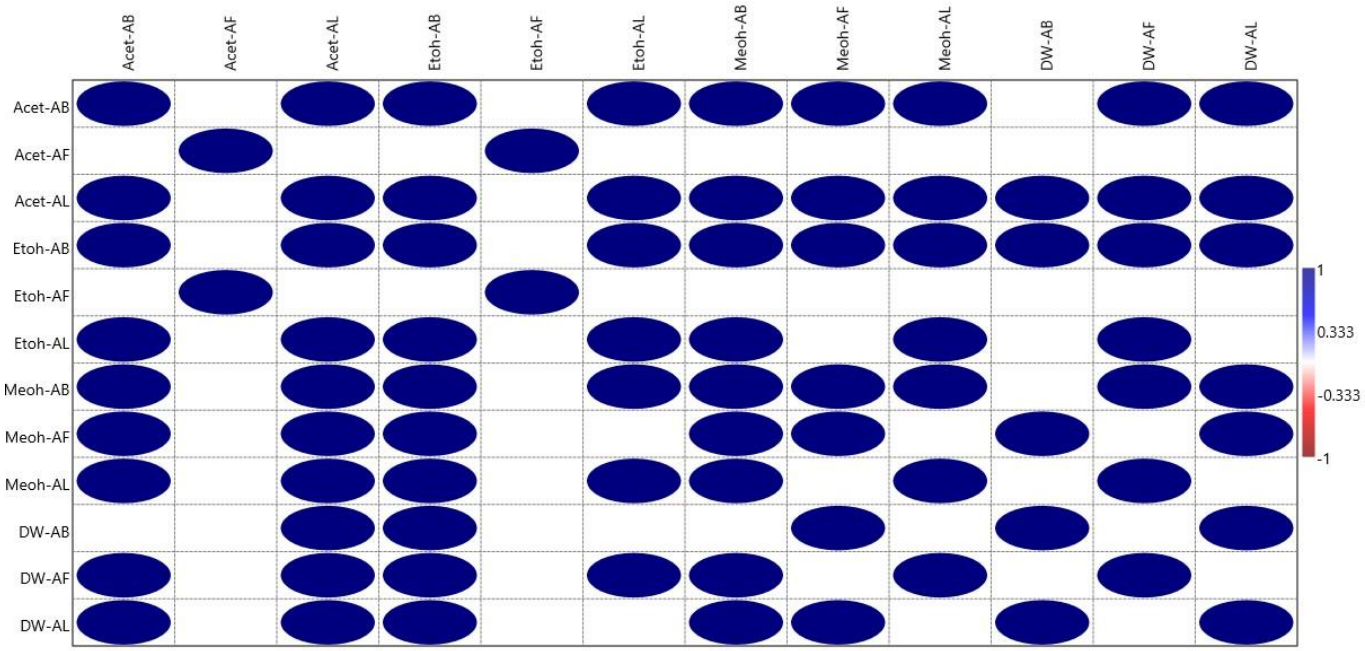

Figure 4. Showing association pattern among solvent extracts based on p≤ 0.05% significance.

Table 4. Showing comparative mineral profiling of individual fractions of *T. arjuna* solvent extracts.

	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	Ga	Se	Rb	Mo	Ag
AF	0.071±0.007	0.006±0.001	0.224±0.004	0.069±0.011	10.815±0.037	0.600±0.025	1.401±0.011	5.145±0.002	29.314±0.001	0.025±0.259	0.152±0.043	0.837±0.022	0.261±0.058	0.017±0.030
AL	0.055±0.009	0.004±.002	0.142±0.007	0.450±0.017	6.837±0.006	0.468±0.032	1.040±0.014	5.214±0.002	23.16±0.002	0.013±0.009	0.084±0.077	0.717±0.025	0.40±0.028	0.009±0.055
AB	0.051±0.010	0.002±0.154	0.115±0.006	0.441±0.017	8.198±0.005	0.373±0.040	1.036±0.014	2.709±0.004	27.394±0.001	0.0141±0.046	0.090±0.072	0.707±0.025	0.158±0.095	0.009±0.055
Br4	0.052±0.010	0.006±0.069	0.166±0.006	0.486±0.015	9.653±0.004	0.437±0.034	1.802±0.008	3.148±0.003	25.114±0.002	0.016±0.040	0.112±0.058	0.775±0.023	0.169±0.089	0.015±0.033
F4	0.032±0.015	0.006±0.069	0.136±0.007	0.417±0.018	6.422±0.006	0.350±0.043	0.665±0.023	3.508±0.003	18.967±0.002	0.0031±0.208	0.060±0.108	0.844±0.021	0.172±0.087	0.014±0.037
L4	0.039±0.013	0.004±0.102	0.137±0.007	0.386±0.019	8.377±0.005	0.326±0.046	0.745±0.020	2.928±0.004	19.307±0.002	0.0113±0.057	0.108±0.060	0.729±0.025	0.148±0.101	0.007±0.0 68
Br5	0.036±0.014	0.004±0.096	0.134±0.007	0.386±0.019	7.570±0.005	0.399±0.038	0.727±0.021	2.940±0.004	22.138±0.002	0.0113±0.058	0.146±0.045	0.687±0.026	0.123±0.122	0.009±0.055
F5	0.042±0.012	0.003±0.141	0.146±0.007	0.427±0.018	6.432±0.006	0.360±0.042	0.675±0.022	3.518±0.003	18.977±0.002	0.0131±0.049	0.070±0.093	0.854±0.021	0.182±0.082	0.024±0.021
L5	0.042±0.012	0.003±0.120	0.176±0.006	0.480±0.016	7.982±0.005	0.411±0.037	1.422±0.011	4.463±0.002	26.411±0.002	0.0131±0.050	0.084±0.077	0.972±0.019	0.186±0.081	0.011±0.046
Br6	0.058±0.009	0.004±0.099	0.155±0.006	0.473±0.016	4.816±0.008	0.471±0.032	1.774±0.008	5.886±0.002	28.523±0.001	0.0159±0.041	0.170±0.038	1.401±0.017	0.033±0.457	0.033±0.015
F6	0.047±0.011	0.004±0.100	0.178±0.006	0.556±0.013	4.830±0.008	0.390±0.039	1.651±0.009	4.868±0.002	45.816±0.001	0.0185±0.035	0.149±0.044	0.874±0.021	0.029±0.523	0.018±0.028
L6	0.063±0.008	0.046±0.009	0.169±0.006	0.439±0.015	5.084±0.008	0.612±0.025	1.871±0.008	4.146±0.003	12.106±0.003	0.0333±0.020	0.130±0.050	0.774±0.023	0.033±0.455	0.014±0.036
Br7	0.074±0.007	0.005±0.008	0.178±0.006	0.273±0.027	7.416±0.005	0.662±0.023	1.890±0.008	4.846±0.002	17.417±0.002	0.0185±0.035	0.111±0.059	0.667±0.027	0.031±`0.478	0.018±0.028
F7	0.056±0.009	0.004±0.091	0.136±0.007	0.464±0.016	8.416±0.005	0.761±0.020-	1.252±0.012	3.885±0.003	19.528±0.002	0.0203±0.032	0.143±0.045	0.860±0.021	0.038±0.393	0.013±0.039
L7	0.048±0.010	0.079±0.005	0.164±0.006	0.470±0.016	9.116±0.004	0.580±0.026	1.356±0.011	4.502±0.002	20.493±0.002	0.0185±0.035	0.130±0.050	0.775±0.023	0.048±0.315	0.013±0.039

Values are Mean± SE, N=3. Here numerical 4, 5, 6 & 7 represent acetone, ethanol, methanol and distilled water and Br, F, and L presenting arjuna bark, fruit and leaf. The comparison was made between avg. yield of individual fraction and avg. yield of serial fraction. AF, AB and AL are being used for arjuna fruit, arjuna bark and arjuna leaf.

Table 5. Showing comparative mineral profiling of individual fractions of *T. arjuna* solvent extracts.

	Li	Be	V	Cr	Mn	Co	Ni	Cu	Zn	Ga	Se	Rb	Mo	Ag
AF	0.076±0.065	0.076±0.002	0.226±0.005	0.697±0.017	11.238±0.002	0.600±0.025	1.387±0.001	5.176±0.004	29.233±0.007	0.019±0.006	0.151±0.040	0.835±0.019	0.262±0.063	0.0164±0.006
AL	0.056±0.018	0.056±0.003	0.1153±0.069	0.454±0.025	6.899±0.003	0.468±0.032	1.027±0.001	5.245±0.004	23.161±0.002	0.013±0.008	0.083±0.072	0.715±0.022	0.541±0.030	0.0087±0.011
AB	0.053±0.019	0.053±0.053	0.166±0.063	0.445±0.026	8.260±0.003	0.373±0.040	1.022±0.001	2.740±0.007	21.399±0.002	0.014±0.008	0.089±0.067	0.705±0.023	0.160±0.103	0.0087±0.011
Br4	0.073±0.014	0.073±0.002	0.201±0.052	0.780±0.015	10.466±0.002	0.472±0.032	1.833±0.001	3.207±0.006	26.319±0.001	0.019±0.006	0.134±0.045	0.785±0.020	0.197±0.084	0.019±0.005
F4	0.054±0.019	0.054±0.002	0.170±0.062	0.711±0.016	7.234±0.003	0.385±0.039	0.696±0.002	3.567±0.006	20.172±0.002	0.006±0.019	0.083±0.073	0.854±0.019	0.200±0.082	0.0171±0.006
L4	0.061±0.017	0.061±0.002	0.171±0.061	0.680±0.017	9.189±0.002	0.361±0.042	0.776±0.002	2.987±0.007	20.512±0.002	0.014±0.008	0.130±0.046	0.739±0.022	0.177±0.093	0.0109±0.009
Br5	0.057±0.018	0.057±0.002	0.169±0.062	0.680±0.017	8.382±0.003	0.434±0.035	0.759±0.002	2.998±0.007	23.343±0.001	0.014±0.008	0.168±0.036	0.697±0.023	0.152±0.109	0.013±0.008
F5	0.064±0.016	0.064±0.003	0.180±0.058	0.721±0.016	7.244±0.003	0.395±0.038	0.706±0.002	3.577±0.006	20.182±0.002	0.016±0.007	0.093±0.065	0.864±0.019	0.210±0.078	0.027±0.004
L5	0.064±0.016	0.064±0.003	0.211±0.050	0.774±0.015	8.794±0.003	0.446±0.034	1.454±0.001	4.521±0.004	27.616±0.001	0.016±0.007	0.106±0.056	0.982±0.016	0.214±0.077	0.0145±0.007

Values are Mean± SE, N=3. Here numerical 4, 5, 6 & 7 represent acetone, ethanol, methanol and distilled water and Br, F, and L presenting arjuna bark, fruit and leaf. The comparison was made between avg. yield of individual fraction and avg. yield of serial fraction. AF, AB and AL are being used for arjuna fruit, arjuna bark and arjuna leaf

Discussion

The multifarious role of *T. arjuna* cannot be overlooked particularly for ethno-medicinal properties. The mechanism behind antimicrobial properties might be due to presence of polyphenolic compounds, and the nutritional and nutraceutical value depends on mineral profiling and biochemical composition concomitant relative yield % and ash content of the compound also. In present experiment the maximum yield for both fractions namely, individual and serial was ascertained in ethanolic bark extracts but did not differ significantly ($p \leq 0.05$) from the methanolic extract of bark as individual solvent and in bark solvent extracts irrespective of solvent systems which is in accordance to Akhter *et al.* [22] who has reported that maximum yield was obtained from ethanolic extract of *T. arjuna* bark. In contrary, to the previous studies [23], where it was pointed out that generally higher extract yields, phenolic contents and plant material antioxidant activity were obtained using aqueous organic solvents, as compared to the respective absolute organic solvents. Further, Ramesh *et al.* [24] revealed that methanolic bark extract showed maximum yield (%) than the other solvents and parts of the *T. arjuna*. In present study the method of extraction was modified from orbital shaking and attempted to receive maximum bioactive principles without affecting the nature and efficacy of the compound and to obtain maximum yield which is corollary to the previous studies [23], where it was revealed that solvent extraction is most frequently used technique for isolation of plant antioxidant compounds. However, the extract yields and resulting antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvent, due to the presence of different antioxidant compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent. In our study the combination of shaking and refluxing was used which was claimed earlier as one of the best extraction techniques for exerting the yield in terms of antioxidant principles and maximizing the yield (%) [23]. In contrast to this some researchers [25] revealed that as far as techniques are concerned, the extracts obtained by the application of sonication demonstrated significant ($p \leq 0.05$) extraction yield and bioactive compounds. The yield (%) and extraction efficiency depend on the nature and polarity of the solvent and material to be extracted. The previous studies conducted in different plant also showed the potency of the ethanol alone or in combination with other solvent systems for maximize the extraction efficiency [26-27]. From Table 1 & 2, a diverse pattern was found in proximate composition particularly for bark which has minimum moisture content and highest ash content in dry powder form in accordance to Amalraj and Gopi [14], who has reported that bark contains 34 % ash, however, it has got maximum moisture and less ash content on encountering the different extraction methods and selective solvents which ascribed the inverse relationship between moisture and ash content of the selected solvent extracts might be attributed to adsorptive nature of ethanol as compared to other solvent systems and subsequently, resulted in lowering the ash content of the ethanolic bark extract. This finding is in partial agreement with Ajazuddin [28], who highlighted in his study that acid-insoluble ash value of the prepared formulation shows that a very small amount of the inorganic component is insoluble in acid. It indicates that adulteration of raw ingredients by substances, such as silica and rice husk, is very less, and a low acid-insoluble ash value may also affect the amount of the component absorbed. Ash content and mineral has linear relationship with fruit extracts and noticeably the methanolic fruit extract showed a surprising trend towards the Zn content which was reported to be as 29 mg/L in dry powder forms and could reach up to 45 mg/L in fractionated forms. Ash and moisture content showed inverse relationship which indicated that fruit extract after extraction had acid soluble ash content more as compared to other solvent extracts which is in accordance to the previous study [29], where it was pointed out that subsequent loss on drying (3.28%), ash content (1.07%), acid insoluble ash (0.26%) were observed in *T. Arjuna* bark extracts. In present study, for both fractions the values of yield, biochemical composition except moisture and ash and mineral profiling did not have much wider variation for a particular solvent extract, however, among the solvent extracts, a diverse patter of variation was observed. *For instances*, there was no relationship between ash and yield % which indicates that ash may be including acid soluble and insoluble content but yield may not deviate with the content of the ash as depicted by the fractions. In this case the maximum yield may be attributed to bioactive compounds such as polyphenols, anti-oxidants etc., which depends on type of extraction solvent and its polarity may have a significant impact on the level of extracted bioactive compounds. The polarities of the bioactive principles range from polar to non-polar, optimum extraction of polyphenols is usually obtained in the polar solvent which have a better efficiency of solvation as a result of interactions (hydrogen bonds) between the polar sites of the antioxidant compounds and the solvent than nonpolar one [30-31]. The mineral profiling also showed same trend for both fractions and particular solvent extract, as mineral profiling of plant material depend on many factors such as nature of tissue extracted, stage of plant used and parts of plant material used for extraction which is corollary to Butkute *et al.* [32], who summarized that mineral concentration of herbal extracts widely depending on the plant growth stage and morphological fraction, and pointed that mineral profile of young plants demonstrated higher content of ash and almost all

elements tested than the flowering one. In the present investigation it revealed that over all mineral profiling was better in terms of methanolic fruit extract which has significant ($p \leq 0.05$) difference with the ethanolic bark extract but no significant ($p > 0.05$) differences with the ethanolic leaf extract, ethanolic fruit extract, acetone fruit extract, and distilled water fruit extracts ,and similarly, acetone although less polar solvent however, its bark extracts has better mineral content as compared to the fruit and leaf extracts and has same pattern of significance as like as other polar solvents which ascribed to type of solvent and nature of material extracted and alteration in bonding pattern of polar aprotic solvents such as acetone which is in harmony to previous studies conducted on other medicinal plants [33-35]. From perusal of Figure 3 the solvent system are assembling in cluster as per the yield (%) concomitant increased polarity and nature of herbal material which ascribed the specific interaction and bonding between solvent and herbal material and dipolar electric point adjustment during solvation which is in accordance to Singh *et al.* [36]. The present study is the first of its kind which is revealing the effective solvent extract with reference to higher mineral profiling and biochemical composition and yield (%) for exploring the possibilities of their inclusion in animal and livestock feed formulation as feed ingredients or as nutraceutical.

Conclusion

From present study some of the novel and innovative results can be highlighted as follows: Solvent extraction with combination of refluxing and orbital shaking could produce better yield ascribing the potency and strength of polar solvents for extracting the biochemical principles in efficient manner. It can be recommended that bark powder can be utilized as supplement for formulating the medicated feed for livestock and fish and methanolic fruit extract to mitigate Zn deficiency and nervous disorders. Although, the mineral profiling was better in methanolic fruit extract, but not-significantly ($p \geq 0.05$ differ from ethanolic fraction, however, the ethanol can be the solvent of choice as far as the toxicity of the solvent is concerned.

Conflicts of interest

The authors declare no conflicts of interest and its original work neither communicate for publication nor under consideration for publication. Further, all authors have contributed to the work immensely.

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