Ultrasound Assisted Vegetable Tannin Extraction from Myrobalan (*Terminalia Chebula*) Nuts for Leather Application

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Abstract

Ultrasound (US) assisted preparation of tannins from *Terminalia Chebula* nuts (myrobalan) through solid-liquid extraction process using water as solvent even dispensing with conventional heating/stirring is presented in this paper. The results indicate there is a significant enhancement in myrobalan extraction due to the influence of US, viz., 1.8 to 4.7-fold (US probe: 20 kHz, 80 W) and 1.2 to 2.2-fold (US tank: 150 W, 33 kHz) in extract %yield and tannin contents respectively as compared to different control processes (static/stirring). Whereas, ultrasound probe at 120 W in 50°C water bath gave the best overall %yield and tanning content. Some of the important scale-up parameters have also been studied and found to be useful, suggesting there could be a significant savings of 427, 184 and 188 kg per ton of raw material usage as extrapolated values based on Lab-scale results in terms of % yield, tannin content and tanning agent offer respectively due to the use of US as compared to control process. The process involves: i). Clean technology with physical activation (US) in aqueous medium at ambient condition – no additional chemicals ii). Green and Eco-benign process with natural materials – useful in all extract applications, iii.) Tannins extract as generic product for better functional properties, iv.) Better diffusion of plant extract through skin matrix in tanning process for leather making, v.) Sustainable solution and eco-conservation through better yields.

Introduction

Bio-active ingredients in the form of tannins are present in some of the plant materials capable of imparting tanning effect. Vegetable tanning is one of the traditional and eco-friendly processes in leather making involving plant materials.[1] Conventional tanning practices mostly involve chrome salts which are not eco-benign. There is a need for green chemistry approach for transforming traditional eco-friendly vegetable tanning technology in to efficient one through the use of modern tools. The approach would not only address the limitations of eco-friendly processes but enhance the functionality of the materials involved. The vegetable tannins are water-soluble polyphenolic compounds having molecular weight in the range of 500 –3000 Daltons.[2,3] Based on their chemical structure, the vegetable tannins are classified as Hydrolysable type (e.g. Myrobalan) and Condensed type (e.g. Wattle). Myrobalan nuts are the unripe fruits of *Terminalia Chebula* tree which are important vegetable tanning material available in India and used in leather making. They contain about 35-40% of tannins which gives mild tanning effect and generally used as supplement in vegetable tanning process and light color to leather. The tannins contain ~14% soluble matter. Tan/non-tan ratio varies from 1.5-2.5. Hydrolysable type tannins are based on esters of phenol carboxylic acid and glucose such as 1,2,3,4,6-pentagalloyl glucose along with several other compounds.[4] The manufacture of the vegetable tanning extract is based on extraction of tannin using a suitable solvent, usually water, followed by concentration and spray drying (to get powder) or vacuum dried (to get solid).[3] Previous reports on myrobalan tannin extraction using ultrasound have not studied important parameters such as tannin content and scale-up aspects. Hence, in the present paper, these aspects are covered with the aim of providing efficient extraction process with better diffusion property through pelt (skin) matrix in tanning process of leather making.

Use of Power Ultrasound

Ultrasound may be broadly classified according to frequency range as power ultrasound (20 to 100) and diagnostic ultrasound (1 to 10).[5] The use of power ultrasound is known to have significant effects on the processes such as cleaning, homogenization; emulsification, sieving, filtration, crystallization, extraction, degassing and stripping etc.[6] Acoustic cavitation in liquid media is mostly responsible for ultrasound induced effects.[7] Moreover, in the liquid phase surrounding the particles, high micro mixing will increase the heat and mass transfer and even the diffusion of species inside the pores of the solid.[6,7] The main advantage of using ultrasound as a physical method of activation instead of chemical methods is that it will not contribute to additional pollution load in the form of chemical entities and also provide possibilities for energy efficient process. Our earlier reports clearly show potential use of ultrasound in leather processing,[8-16] vegetable tannin extraction from myrobalan[8] and wattle.[17] Natural dye extraction from beetroot[18] and flowers[19] and have also been reported.

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Manuscript received October 9, 2017, accepted for publication October 31, 2017.

In comparison with our previous available literature in the particular subjet,[8] several important aspects such as: 1) preparation of tannins from myrobalan, 2) the effect of US on tannin content, 3) up-scaling parameters, 4) uptake kinetics of tanning extract in pelt matrix, 5) experimental results and process scale-up considerations have been studied in this paper.

Experimental

Experimental Set Up

Ultrasound (US) assisted extraction experiments were carried out using ultrasonic tank, UST (*Roop-Telsonic, Ultrasonics, India Ltd.*), generating ultrasound at 33 kHz and 150 W power (fixed) as quoted by the manufacturer.[15,20] In order to vary the power, ultrasonic probe, USP (*VCX 400, Sonics and Materials, USA*, 20 kHz & 0-400 W) in a jacketed glass vessel with provisions to set required output power and time was also used.[8] Control experiments were carried out in a water bath with provisions to control temperature as static control (SC) as well as magnetic stirring (MS).

Experimental Procedure

Myrobalan nuts of Indian origin were broken in to average size of ~0.5 cm. Extraction was carried out using water as a solvent with suitable ratio, typically 1 g of nuts in 30 ml water. Experiments with ultrasound were carried out and compared with those not using ultrasound, in stationary as well as magnetic stirring condition. Tannin extraction is carried out using ultrasound. Influence of process parameters such as ultrasound power, time, temperature and amount of materials, solvent ratio were studied. Two-stage extraction has also been studied in view of process scale-up.

Tanning Trials

The effectiveness of myrobalan extract obtained using ultrasound in leather tanning process has been studied by conducting tanning trails. For this purpose, pickled buffalo pelt with pH ~ 5-5.5 has been taken for the tanning trials. Two sample pieces (E & C) with 5´5×cm sizes have been cut from the butt portion of the pelt parallel to the backbone. The weights of the two samples were recorded individually and the experiments done with duplicates. For tanning purpose, 20 g nuts in 120 ml water were sonicated using USP, 120 W as compared to static control for 6 h. Then the pelts were treated separately with 20% (*w/w*) of tannin extract (*on pelt weight basis*) using tanning extract solutions (*w/v*) from ultrasound extract (UE) and control extract (CE). The pelt along with tannin solution were taken in a 250 ml conical flask and agitated in a Remi shaking machine with a speed of ~180 strokes/minute.

Analytical methods

% Extract yield

Every 30 minutes, samples were taken from both ultrasound and control extracts in clean, dried and weighed glass dishes. The extracts were dried in a hot-air oven till all the water evaporated and only the extract was left. The dishes were then cooled in a desiccator and weighed. The drying, cooling and weighing procedure was repeated to get the constant weight and the weight of the extract was determined. The weight of the extract obtained per gram of the nuts used was calculated. The yield has been calculated using the equation,

$$\%\text{Yield} = \frac{\text{Extract obtained } (g)}{\text{Amount of nuts used } (g)} \times 100 \tag{1}$$

Enhancement factors for myrobalan extraction with ultrasound (ξ_{us})

The influence of ultrasound on myrobalan extraction can be defined with two different enhancement factors as α and β (Eq. 2 and 3),

α - Enhancement factor for total extraction

β - Enhancement factor for tannin content in the extract

$$\alpha = \frac{Y_{us}}{Y_c} \tag{2}$$

Where, Y_{us_power} – Yield of ultrasonic process for set power at time (t)

Y_c – Yield of control process at T°C for comparison (SC or MS) at time (t)

$$\beta = \frac{Y_{us-\tan nin}}{Y_{c-\tan nin}} \tag{3}$$

Where, Y_{us_tannin} – Yield of tannin for ultrasonic process for set power at time (t)

$Y_{c\text{-}tannin}$ – Yield of tannin for control process at T°C for comparison (SC or MS) at time (t)

Quantitative Analysis of Tannin Content

The Folin-Ciocalteau method of analysis of tannin was used to estimate the tannin content of the extract as described.[20] Gallic acid was used for the preparation of the standard graph, since it acts as a building block in the formation of hydrolysable tannin material. Tannins react with the Folins reagent to form a colored complex and the solution was then analyzed using UV spectrophotometer for the absorbance at 760 nm. Then amount tannins are expressed in terms of Gallic acid equivalents.

Results and Discussions

Myrobalan Extraction in Ultrasonic Tank

Extraction in ultrasonic tank was carried out using 1 g myrobalan nut in 30 ml water. The results indicate that there is a significant 1.9-fold improvement in extract yield due to the use of US as compared to static control at the end of 6 h as shown in Figure 1. Whereas, there is a 3.3-fold increase in tannin content as shown in Figure 2.

Effect of Extraction in US Probe

Effect of extraction in US probe, 80 W using 2 g nuts in 60 ml water has been studied and compared with process without US at 30°C and 40°C. There is a 1.7 and 1.12-fold increase in extract yield due to US, 80 W as compared to static control at 30°C and 40°C respectively as shown in Figure 3. Whereas, there is a 1.4 and 1.2-fold increase in tannin content respectively as shown in Figure 4.

Effect of US Power, Temperature and Stirring in Extraction

Effect of US power (80-120 W), temperature for water bath (WB) static control (30-60°C) and magnetic stirring (MS) in extraction of 20 g nuts in 120 ml water has been studied. The results indicate that there is a significant improvement in both extract yield and tannin content due to US, 120 W as compared to different control processes for 6 h time is shown in Figure 5 and 6 respectively. The order of improvement in %yield has been found to be US, 120 W > US, 100 W > US, 80 W > WB, 60°C > WB, 50°C > WB, 40°C > MS > WB, 30°C. Whereas, the order for tannin content being US, 120 W > US, 100 W > WB, 60°C > US, 80 W > WB, 50°C > WB, 40°C > WB, 30°C > MS.

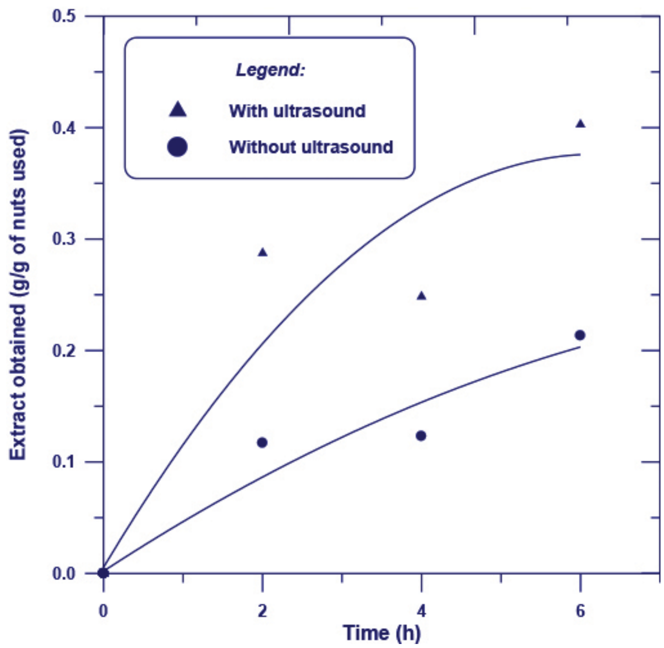

Figure 1. Effect of ultrasound (US tank) on the total extract obtained using 1 g of nuts in 30 ml of water.

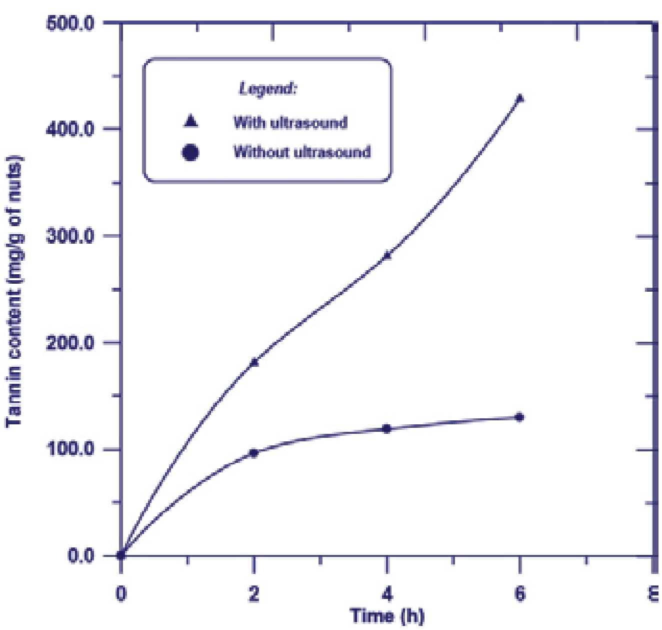

Figure 2. Effect of ultrasound (US tank) on the tannin extraction for 1 g of nuts in 30 ml of water.

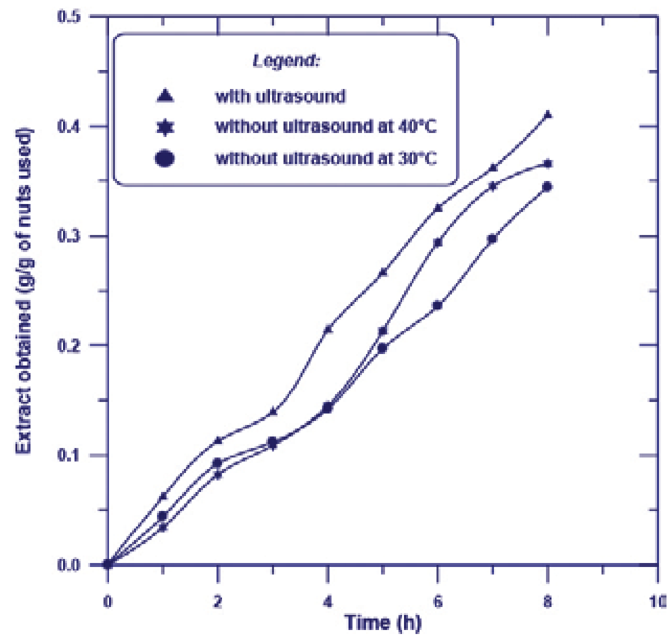

Figure 3. Effect of ultrasound (80 W) on the extraction efficiency for 2 g of nuts in 60 ml of water.

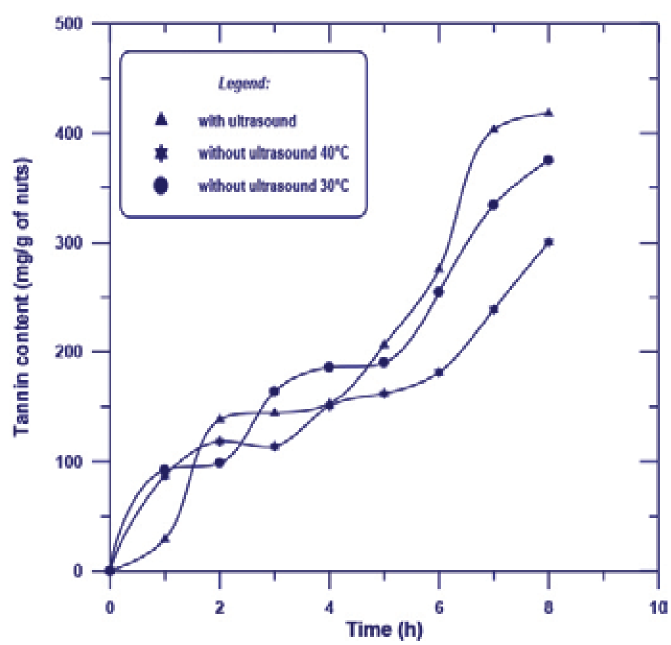

Figure 4. Effect of ultrasound (80 W) on the tannin extraction efficiency for 2 g of nuts in 60 ml of water.

Enhancement Factors (ξ_{us}) for Myrobalan Extraction with Ultrasound

ξ_{us} for myrobalan extraction with ultrasound (120 W) for both total yield (α) and tannin content (β) are calculated as compared to different control (SC and MS) processes (Eq. (2) and (3)) for 6 h time and given in Table I. The results indicate that there is a considerable improvement in ξ_{us} values such as *a* and *b* due to US, 120 compares do different control processes such as SC and MS at different temperatures.

Effect of Distance of Material from US Probe Tip

Effect of distance (*d*) of material from US probe tip, another important parameter for the US assisted extraction as *d* – 1, 10, 20, 30 and 40 mm has been studied using 1 g nuts in 50 ml water at 80 W power. The results indicate that there is a substantial improvement in %yield > 50% for *d* = 1 as compared to other distances as shown in Figure 7. Therefore, this result is very important as maximum ultrasound cavitation intensity available near the US probe tip, the solid-liquid extraction process with ultrasound is significantly affected by ultrasound induced cavitations.

Mechanism tor The Ultrasound Assisted Extraction

Following steps could be involved in the ultrasound assisted Extraction,

Step 1: Flow of solvent at high speed on plant material i.e. myrobalan nuts due to microjet formation from ultrasound effects

Step 2: Erosion and breaking of plant cells due to ultrasonic cavitation effects

Step 3: Dissolution of active ingredients present in the plant material such as tannins in to bulk solvent

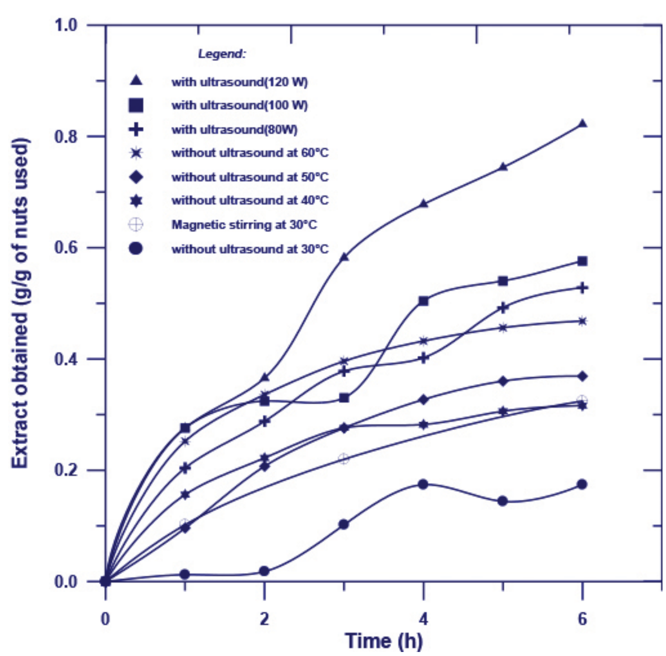

Figure 5. Effect of ultrasound on the extraction efficiency for 20 g of nuts in 120 ml of water.

Step 4: Further break down of tannins in to smaller fragments

Step 5: Effects of ultrasound (*Step* 1-4) continue on fresh plant surfaces created

Two-stage Extraction

First and Second stage extraction carried out with extract solution carry over from first batch stage to second stage using 100 ml water with 1 g nuts in each stage, resulted in % yield of 45 and 90 respectively for 2 h as given in Table II. Similarly, %yield 45 and 85 was obtained using 2 g nuts in each stage with extract solution carry over for 2 h. Therefore, the results indicate there is a significant improvement in concentration of extraction solution with 85-90% concentrations, useful in spray drying stage in up-scaling process.

Efficacy of Extract in Tanning Process

Efficacy of extract obtained using ultrasound and control was checked in tanning process as described in previous section. The %exhaustion of tanning agent was calculated for both UE and CE as shown in Figure 8. The results indicate that there is a significant improvement in %exhaustion of myrobalan tanning agent for UE as compared to CE. Based on our earlier studies on this subject, the reason could be due to enhanced diffusion of tannins through the pelt matrix provided by considerable attenuation of particle-size of extract in one hand and reversible pore-size changes in pelt matrix on other.[1,14]

Experimental Results and Process Scale-up Considerations

Ultrasound usage and subsequent effect of tannins provide significant improvements in %yield, tannin content and %exhaustion of tanning agents as compared to control process useful in bio-conservation of natural materials. The results

Table I

Enhancement factors, (ξ_{us}) for %yield (α) and tannin content (β) due to the use of US, 120 W in 120 ml water as compared to different control processes for 6 h time.

Enhancement factor for %yield (α)				
Static control				Magnetic stirring
30°C	40°C	50°C	60°C	30°C
4.7	2.6	2.2	1.8	2.5
Enhancement factor for tannin content (β)				
Static control				Magnetic stirring
30°C	40°C	50°C	60°C	30°C
2.0	2.0	1.3	1.2	2.2

indicate there is a significant 1.8 to 4.7 using (US probe: 20 kHz, 80 W) and 1.2 to 2.2-fold (US tank: 150 W, 33 kHz) improvement in myrobalan extract %yield and tannin contents respectively due to the use of US as compared to different control processes (static/stirring). The order of improvement in %yield has been found to be US, 120 W > US, 100 W > US, 80 W > WB, 60°C > WB, 50°C > WB, 40°C > MS > WB, 30°C. Whereas, the order for tannin content being US, 120 W > US, 100 W > WB, 60°C > US, 80 W > WB, 50°C > WB, 40°C > WB, 30°C > MS. For every tone of raw material processing, there could be a significant savings of 427 and 184 Kg in raw material usage in terms of %yield and tannin content respectively due to the use of US as compared to control at 60°C as shown in Table III. Whereas, in the case of tanning process improvements in %exhaustion due to the use of tannins could bring about significant savings of 188 Kg of tanning materials as compared to control tanning extract. The improvements in tanning process would also minimize considerable amount of un-used tanning agent in spent liquor and subsequent reduction in pollution load.[28-29]

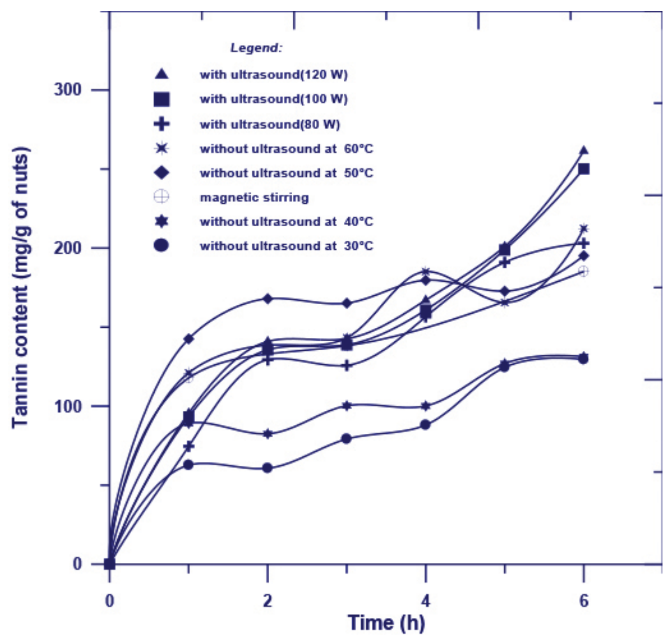

Figure 6. Effect of ultrasound on tannin extraction efficiency for 20 gm of nuts in 120 ml of water.

Table II
Two-stage extraction with extract carry over using ultrasound, 80 W.

Experiment	Time (h)	% Yield	% Yield
		Extract carried over with fresh material	Extract carried over with fresh material
		1 Batch	2 Batch
(1 g × 2) in 100 ml	1	40	85
	2	45	90
(2 g × 2) in 100 ml	2	45	85

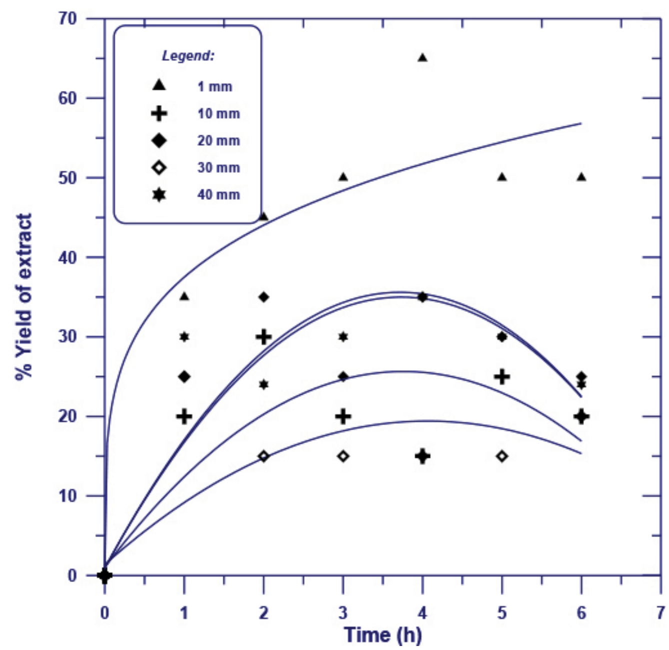

Figure 7. Effect of distance (d – 1, 10, 20, 30 and 40 mm) of material from US probe tip in US assisted extraction using 1 g nuts in 50 ml water at 80 W power.

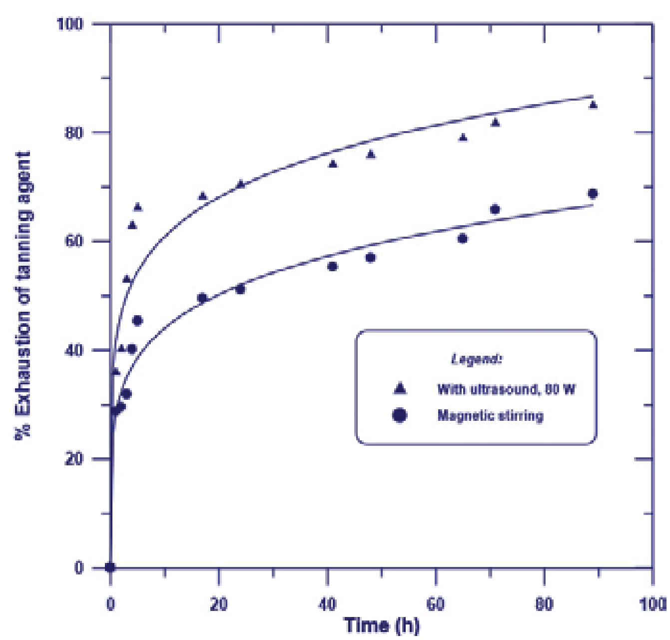

Figure 8. Effect of extract prepared using ultrasound (20 g nuts in 120 ml water, USP-120 W) as compared to static control in tanning with 20% (*w/w*) of tannin extract (*on pelt weight basis*).

Table III
Savings in raw material usage in Kg due to US Vs C for processing 1 T of the material in terms of various parameters.

S. No	Parameter	Ultrasound process (US)	Control process (C)	Savings in raw material usage in Kg due to US Vs C for processing 1 ton of the material
1	%Yield	82	47 (for 60°C)	427
2	Tannin content (Kg/t)	261	213 (for 60°C)	184
3	%Exhaustion	85	69	188

Conclusions

Ultrasound assisted leaching of *Terminalia Chebula* nuts facilitate the formation of tannins and provided significant improvements in yield, tannin content useful in bio-availability as well as bio-conservation. From the experimental results, ultrasound probe at 120 W in 50°C water bath gave the best overall %yield and tanning content. These tannins have been found useful for better diffusion, uptake and distribution of the extract through the pelt matrix in the tanning process for leather making. Some of the parameters for a process scale-up have also been studied and considered to be a useful suggestion. Therefore, to conclude, tannins prepared from this novel ultrasound method offers a potential natural material for a variety of applications for sustainable development and eco-conservation, and not only limited to tanning process alone.

Acknowledgements

One of the authors (V.S) thanks CSIR-CLRI & Council of Scientific and Industrial Research (CSIR), India, for financial support.

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