Extraction, Screening and Spectral Characterization of Tannins from *Acacia Xanthophloea* (Fever Tree) Bark

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Abstract: Identifying studied sample acacia species selected and collected from Bekele Molla Hotel Avenue, Chamo Kebele, Arba Minch under convenient random sampling method. Tannins extracted using the solvents such as water (at 60 & 90⁰C), methanol (at 90⁰C) and its 1:1 mixture (at 90⁰C) by employing Soxhlet extraction. Extracted crude tannins was separated and purified under vacuum distillation. The photochemical screening and quantitative determinations of sample carried out. Furthermore, the sample of tannins characterized by employing U-visible and FTIR spectroscopic studies. Methanolic extract consist moisture content and % yield about 17.86±0.51 % and 52.55 %. The results of Uv-visible and FTIR spectroscopic studies were supporting that the sample of *Acacia xanthophloea* has tannins and flavonoids. Thus, this study reveals and confirmed that the methanol is more suitable solvent for extraction of tannins over than water.

Keywords: *Acacia xanthophloea*, *Fever Tree*, *FTIR spectroscopy*, *Methanol*, *UV-visible*

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1. BACKGROUND OF THE STUDY

Raw skin/hide is a stuff which is simply degradable, but through the serious step of processing including tanning of raw skin/hide changed into physico-chemically stable leather, even that exposed into a series of different processes. Upon the processing, where the skin becomes stabilized and its hydrothermal strength improved are communally called as tanning [1, 2]. Ancient day onwards leather processing from animal hides undergone; but, till now the modern leather processing uses chromium for tanning of the skin. Chrome tanning is harmful to the environment; due to discharging off some harmful chrome salts they have produced with little skill and very often mass products during tanning process. Chrome tanning is by far the most commonly used method for tanning of leathers and shoe upper leathers. The advantages

of chrome tanning materials over the other commercially available tanning materials are the

high speeds of protein fixation with low chemical costs, and excellent hide preservation but products used in processing fails about the impact on the environment. Further, the characteristics of an industrial wastewater stream depend to a large extent on the type of products being produced and treatment of pollutants is also on the added expensive for the industry [3]. Thus, avoid the environmental pollution and the strict environmental regulations implemented by the developed and developing nations. The leather industries and researchers forced to find alternative tanning materials from natural resources. Hence, the researchers undertaken the series of researches and found the plants (phyto) based tanning materials called bio-tannins or vegetable tannin that used successfully in many researches and proved as alternative to the

chromium that used in chrome tanning in leather industries [3]. The corresponding tanning of skin/hide that transformed in to leather by undergone with bio-tannins called as bio-tanning or vegetable tanning (also termed as phyto-tanning).

Tannins (hydrolysable and non-hydrolysable) have capability to make linking with skin-proteins (collagen) form compact and hydrological steady product called leather. Tannins are well-known ingredient in the process of tanning of leather. According to the literatures stated [4-8]. Tanbark from oak, Mimosa, Birch, Hemlock, Chestnut, okra, Fir bark and Quebracho (have catechol) and Oak bark has both types of tannins. All the phyto-tannins have traditionally used as the primary source of tannery tannin, and account for 90% of the world's leather production.

Phyto-tanning engage treats the hides and skins with vegetation and bark restrain tannins. Tannins are polyphenolic compounds with molecular weight of 500-20,000, makes precipitate of proteins and alkaloids. Although the tannins make crosslink with collagen and it's develop leather. But, quality of leather affected (by type and sources of tannins) during pre-tanning and post-tanning processes, and corresponding tanned matters can influence to the value of leather.

The type of tanning materials used in leather production affects the physical characteristics of the leather which produced from the same origin. Although, phyto-tanned leathers offers some physico-mechanical benefits to the finished leather [9, 10].

Skins and hides tanned with phyto-tannins from different sources of tanning materials gives leathers with different physical properties. The physical properties of final leather find its uses on preparation of the product. All the vegetable tanned leathers for use in making leather products must meet the minimum recommended standard to make satisfactory products of good quality [11]. Ekaterini Malea *et al.* [12] extracted the tannins from four native and foreign woody plant parts such as leaves, bark, stem and roots studied and characterized by influences of different parameters.

Phyto-tannins that almost used to tan any type of hide and skins of cows, calves, buffalos, goats, snakes, sheep, pigs, ostriches, and stingrays [13]. The reported results shown, the plant-based tannins are better alternative method of tanning to

chrome tonnage because of environmental friendly than chrome tanning, low-cost and ease to apply in to diverse kinds of leather.

Unlike chrome tanning, vegetable tanning can take up to at least 3-4 days to produce a piece of dyed leather. But, it is environmentally friendly and recyclable, only natural ingredients could be used when dyeing the hides, better performance leather might be achieved often healthier than chrome tanning [6-12]. In this concern, *Acacia xanthophloea* (*A.xanthophloea*) is one of the most important vegetable tanning material, which suggesting alternative bio-tannin raw material for getting finished leather. This variety of acacia tree (shown in Figure 1) is easy accessible in study area.

Despite that the widely available local resources like *A.xanthophloea* like plant bio-materials and vegetable tanning remains underdeveloped and neglected method of leather tanning in Africa. But it's a very important to health concern, to cut economic expenditure and reduces the hazardous to existing life and environment.

In this context, the current study focused the characterization of bio-tannins that extracted from *A.xanthophloea* bark. These kinds of bio-tannins are mainly useful raw materials into leather industries as vegetable tanning that are also used as natural ingredients available around us. Furthermore, vegetable tanning is arguably more sustainable and ecologically friendly than conventional leather tanning methods, especially chrome tanning method.

Figure 1. Photograph of *Acacia xanthophloea* tree

2. EXPERIMENTAL DETAILS

2.1 Preparation of *Acacia Xanthophloea* Bark Powder

Matured bark part (Figure 2) of *A.xanthophloea* was collected under convenient random sampling method from Bekele Molla, Chamo Kebele, Arba Minch. The collected samples were pre-treated as by cleaned and washed well with water and left for air dry under room temperature and dried in sunlight. The pre-treated samples were grinded by using laboratory analytical mill grinder and obtained a crude powder (Figure 3). Then the powdered plant samples were preserved under moisture free atmosphere for further use to extract the tannins under room temperature.

2.2 Preparation of Crude Tannin Extract

Bulk collections of bark samples (dried in air) grounded well in a Lab mill and sieved through 2 mm sized sieves. About 30 g part of the sample placed in a 250 mL thimbles of Soxhlet apparatus (Figure 4) with 150 mL of distilled water, another (30 g) part of the sample with 80 % methanol, and a third part (30 g) sample placed with 1:1 methanol and water mixture (250 mL) each. The solution subjected to heat for 4 hours with constant temperatures at 30°C, 60°C and 90°C for extraction of tannins and for other portions carried out at 90°C for 4 hours. All the samples filtered (using Whatman No.1 filter paper, 18.5 cm disc) and the remaining stuff washed and separated with more solvent. Extracts had transferred in to a tarred, volumetric flask and concentrated by employing vacuum rotary evaporator (Figure 5), obtained concentrated extracts. Samples of extract desiccated in a vacuum hot air-oven at 60°C [14], achieved bright reddish-orange solid/powder (shown in Figure 6) that used for further experiments.

2.3 Characterization of *Acacia xanthophloea* Tannins

Characterization of crude extract done by determination of moisture content, percentage yield, types of tannins (condensed type or hydrolyzed), amount of tannin or total phenol, total flavonoids, total solids, as well as color of the tannin present in the selected plant of *A.xanthophloea*. In order to estimate the composition of tannins of the sample, it's characterized through UV-visible and FTIR spectroscopic studies, and compared the results with reported literatures of tannins and related phenolics.

2.3.1 Determination of Percentage Yield of Extract

Extract of each samples (3 samples) loaded on a pre-weighed beaker, and kept in an oven about 105°C until reached constant weight, cooled to room temperature; make sure free from any trace of moisture by employing desiccators containing CaCl₂ and weighed the sample again.

The extract of each sample allowed and reacting efficiently with 20 mL of 1:1 v/v solvent mixture of con. HCl and formalin (1:1 v/v) for 4 hours, that enable the solute to dissolve in it. The precipitated solution filtered through a pre-weighed sintered glass filter, washed then with warm water. Obtained precipitate air-dried to 10% moisture and reweighed. Percentage yield of sample calculated using the formula [15].

$$\% \text{ yield of sample} = \frac{W_2 - W_1}{W_o} \times 100$$

Where,

W_o - Initial weight of sample

W₁ - Weight of empty beaker

W₂ - Weight of the extract + beaker after dried the sample

2.3.2 Moisture Content

About 5g of finely grounded extracted tannins of each sample placed in a pre-weighed crucible and set aside back in hot air-oven at 90°C up to 3-4 hours (achieved stable weight), slow down the temperature of the crucible in to room atmosphere by keeping in desiccators and reweighed. Then, moisture content was estimated from the weight loss of the sample [15]:

$$\% \text{ moisture} = \frac{\text{Loss in weight (w}_1 - w_2)}{\text{Weight of sample (w}_1)} \times 100$$

Where,

W₁ - Weight (g) of the sample taken for test

W₂ - Weight (g) of residue left after drying

2.3.3 Identification of Tannins Using FeCl₃

About 5 drops of alc.FeCl₃ is added into 1 mL of the aqueous sample taken in a test tube. Green to black precipitate might be appears, it reveals the presence of tannins according to literature [16].

2.3.4 Test with Ferrous Sulphate Solution

Accurately measured part (1 mL) of the sample extract taken place in a test tube and 0.1 % FeSO₄ (2 mL) and 0.5% sodium potassium tartarate

Figure 2. Photograph of *Acacia xanthophloea* tree and bark part

Figure 3. Powdered crude extract of *A. xanthophloea*

Figure 4. Soxhlet apparatus used for the extraction of *A.xanthophloea* tannins

Figure 5. Rotary evaporator used for the *A. xanthophloea* tannin extraction

Figure 6. Extracted, powdered & crystallized *A. xanthophloea* tannins

added into the test tube containing sample. Appearance of violet color indicates presence of tannins as evident through reported [17].

2.3.5 UV-Visible spectral Analysis of *Acacia xanthophloea* Tannins

Exactly weighed (250 mg) measure of sample powder dissolved in distilled water (50 mL) and filtered it. Then, analyze filtrate through Uv-visible spectroscopy (SPECORD 50 PLUS, Germany) at a ranged 190 to 600 nm at an interval of 20 nm against water blank, and detected the characteristic wavelength of the studied plant extract.

2.3.6 FTIR Spectroscopy Analysis of *Acacia xanthophloea* Tannins

The dried grinded fine powder of samples mixed (1:100 ratio) with KBr (FTIR grade) and hard-pressed in to a pellet. It's kept instantly into the sample tube of Spectrum 65 FTIR (Perkin Elmer) and scanned in the range 4000-400 cm^{-1} at room temperature. The spectrum of the samples and its data is obtained by using essential FTIR software. Obtained spectral data and the functional groups detected that compared with the earlier reported studies.

3. RESULT AND DISCUSSION

3.1 Determination of Moisture Content

Moisture content analysis gives the information of moisture available in the given sample of *A.xanthophloea* bark. The result of moisture content of the sample is 17.86 ± 0.51 % by weight of the sample and comparison of the moisture content on triplicate measurement is closure and standard deviation become ± 0.51 , which shows the accuracy of measurement (shown in Table 1). Thus, the sample of *A.xanthophloea* have greater amount of phyto-chemicals with less moisture content.

Table 1. Determination of moisture content on sample *Acacia xanthophloea*

Trials	Weight of extract before drying (g)	Weight of extract after drying (g)	Moisture content (%)
1	5.21	4.275	17.95
2	5.072	4.194	17.31
3	5.152	4.208	18.32
Average $\pm$ SD (g)			17.86 ± 0.51

3.2 Estimation of % Yield of Tannin Extract

In this study, the soxhlet extraction of tannin from *A.xanthophloea* bark carried out by employing two solvents such as water and methanol and its mixture (1:1) with in a different temperature (30-60°C for water; 90°C for water and mixture of solvents). After the extraction process the solvents removed by two techniques as water removed by evaporation by using hot plates along with direct heat since methanol removed by using rotary evaporator. Results of extraction were given in Table 2. While comparing the results of % tannin extraction, aqueous extract at 90°C has greater yield (49.22 %) than extraction of tannins at 30 and 60°C (shown in Table 2). Also, % yield of tannin extraction compared upon different solvents, the results were reveals that the methanol used as a extracting solvent that extract has the greater amount of tannins (52.55% of yield) from the sample of *A.xanthophloea* than other solvents such as water and its mixture (shown in Table 2). This is due to the solvent that had superior percentage of methanol; even though tannins are well-known highly water-soluble. Hence, water proved as an ineffective extracting solvent for tannins. This is due to the formation of tannin-protein complexes. The efficiency of methanol as a solvent used for extracting of tannin might be due to it being an organic polar solvent. The methanol polarity makes it able to have strong interactions with polar substances like tannin [18].

From this study, bark of *A.xanthophloea* is identified as a source of vegetable tanning material. Although the local tanners are not aware of the tannin content, and this study suggests that the barks which produces deep colored liquor has more tanning chemicals compared to other plants parts according to Smith et al. [19]. They also found that the barks from acacia species have more tannin content than other plants. *A.xanthophloea* tannins has not been used in leather production but used as medicinal plants.

This study found that the percentage of tannins content in methanol, water at 30°C, 60°C and 90°C and mixture of water and methanol of *A.xanthophloea* bark as 52.55 %, 37.22 %, 39.22 %, 49.22 % and 45.01 % respectively. The findings of this study differed slightly with findings from other studies elsewhere.

Table 2. Yield of *Acacia xanthophloea* Tannin Extract

Solvents	Temperature (°C)	Amount of tannins extracted (g/30g of sample)			Average (X±SD) (g)	% of extraction
		Trial-1	Trial-2	Trial-3		
Water	30	10.2	11.2	12.1	11.166±0.95	37.22
	60	10.7	11.6	13.0	11.766±1.16	39.22
	90	13.6	12.6	18.1	14.766±2.93	49.22
Methanol	90	19.2	17.1	11.0	15.766±4.26	52.55
Methanol & Water (1:1)	90	13.01	13.9	13.6	13.503±0.45	45.01

For example, a study has reported by Nundkum and Ojewole [20] in South Africa have found *A.xanthophloea* barks would contain around 50% condensed tannins in methanol solvent and 36% in water solvent extracted. The differences in the tannin content from the various studies found that might be due to the variations in the environmental factors, high temperature, water stress, extremes light intensities and poor soil quality are known to increase the tannins contents in plants. The tannins contents also vary with the bark thickness, age of the trees and from the base of the trunk upwards with the branch having low tannin contents [21].

Haroun *et al.* [22] reported that the quantitative data revealed that the barks of the trees species have more than 35 % tannins required for commercial extractions, that's confirmed from the study, methanol could be used as more efficient extraction solvent than water and methanol -water mixtures (Table 2) in terms of the mass of tannin substance extracted from a given mass of studies sample *A.xanthophloea*.

3.3 Tannin Screening Test

Tannin screening test of the extracted samples carried out and the results show that presence of some of the polyphenolic phyto-chemicals as per literature stated [23]. The noticed observation is given in Table.3.

Table 3. Chemical Tests of the extracted samples of *Acacia xanthophloea*

Test	Water extract	Methanolic extract	Mixture of methanol + water
Ferric chloride	positive	positive	positive
Ferrous sulphate with sodium tartarate	positive	positive	positive

The results shows that while undergone the sample of extract with chemical tests does obtained positive results, reveals that presence

of tannins in a studied sample of extract. Also the extracting solvents such as water, methanol and its mixture as well confirmed to extract the tannins from bark of *A.xanthophloea*.

3.4 Spectral Studies of *Acacia Xanthophloea* Tannins

3.4.1 UV-Visible Spectroscopic Study

3.4.1.1 Characterization of Aqueous Tannins Extract on Temperature

The UV-visible absorption spectrum of the aqueous tannin extracts that obtained upon different temperatures shown in Figure 7. The present study reveals the major bands which were appears in the range of 270 -320 nm with the absorbance about 0.39 - 1.94. The spectrum for concern polyphenols typically lay in the range 230-290nm that reported [24]. Kajdzanoska *et al.* [25] revealed that the absorption maxima at 280 show flavonols. Sisa *et al.* [26] reported that the absorption maxima at 255, 280, 330 and 350 nm shown the presence of flavone ring system. Thus, resulting UV-visible spectral study authenticate the existence of tannins and flavonoid ring system on aqueous extract of studied *A.xanthophloea* at all range of extraction temperatures between 30°C-90°C.

Figure 7. UV-visible spectrum of *Acacia xanthophloea* tannin on water at 30 - 90°C

Furthermore, compare the UV-visible spectrum of water extract samples upon 30°C and 90°C are almost similar beyond the

wavelength of 260 nm then later slightly lower absorption of the spectrum of lower temperature (30°C) fraction of extract. But, at lower range of wavelengths the spectrum of tannin sample (extract at 30°C) has higher absorption, due to may decompose some phyto-chemicals upon rising the temperature moderately but not at high temperature close to boiling point of water (shown in Figure 7). Also, extracted the tannins from *A. xanthophloea* at 90°C has greater yield that confirms the maximum absorption at all ranges of wavelength and existing the shifting of $\lambda_{\max}$ towards higher wavelength called bathochromic (red) shift. But in all the cases there is no change does takes place at 506 nm, show that the corresponding phytochemical persist consistently (Figure 7) without decomposition at all temperature ranges.

3.4.1.2 Effect of Solvent for Tannins Extraction Using UV-Visible Spectra

UV-visible spectrums of tannins extracted from *A.xanthophloea* by employing different solvents presented in Figure 8. This reveals that appearance of peak in respective wavelengths is similar like the peak of spectrum corresponds to the extracted samples on aqueous medium. The spectrum of the extracts compared with standards when using different solvents such as methanol, water and its 1:1 mixture that shows peak maxima ranged at 292-296 nm by decreasing absorption of respective $\lambda_{\max}$ peak, this trend followed throughout the range of wavelength. It shows that extraction of tannins in water has largest yield that get decreases by employing the extraction with methanol and mixed methanol does exists hypsochromic (blue shift) and hypochromic shifts (decrement of absorption) that shown in Figure 8. Absorption peak maxima around 280-296 nm and 252-262 nm and compared with standards of polyphenols show presence of polyphenolic acids (shown in Table 4), and catechin (Flavan-3-ols) considered as a monomer of condensed tannins as reported literature [27]. The results revealed that the water may consider as an appropriate solvent than methanol and its mixture.

3.4.2 FTIR Spectroscopic Study

FTIR characterization of the presently studied samples and its corresponding peak picking spectrums described in Figures 9 and 10. The study revealed that the strong absorption band

between 3700-3100 cm^{-1} with a broad & well-built band. It shows, presence of -OH stretching and owing to broad variety of hydrogen bonding between hydroxyl groups, which is well agreed with literature [28] and showing availability of phenols and alcohols. Band at 2926 cm^{-1} and a little shoulder around 2850 cm^{-1} correlated with symmetric and anti-symmetric CH- stretching's of CH_2 and CH_3 groups. Bands in the region 1600-1400 cm^{-1} corresponds to C-C stretching, confirm existence of aromatic compounds and absorption band region of 1500-1400 cm^{-1} shows deformation of C-C bonds of the phenolic groups present in the studied sample of *A.xanthophloea* tannins.

Figure 8. UV-visible spectrum of *Acacia xanthophloea* tannin on different solvents.

Table 4. UV spectral data of standards of polyphenols and sample *A.Xanthophloea* (bark)

Tannins and related phenolics	Wavelength (nm)	
	Ethyleneic band (E_2 - band)	Benzoic band (B - band)
Std. gallic acid	216	264
Std. tannic acid	220	280
Std. catechin	208	280
Std. catechol	216	276
Std. m-hydroxy benzoic acid	207	292
Sample of <i>A.xanthophloea</i> (bark)	250	252-262 280-296

Peak around 1320 cm^{-1} correspond to C-O stretching which confirms the occurrence of alcoholic, carboxylic acid, ester and ethers [34] and peak at 1616 cm^{-1} represents aromatic -C-O stretching. Aromatic $\text{CH}_2\text{-OH}$ (- CH_2) vibration is identified by the peak appear at 1443 cm^{-1} . IR spectrum of presently studied samples does not shows any signals of free H_2O molecules owing to nearby no supportive

bands at 2130 cm^{-1} & 1650 cm^{-1} and at about 700 cm^{-1} [29].

Figure 9. FTIR spectrum of *Acacia xanthophloea* tannins on water at 60°C and 90°C

The FTIR spectrum of the study also shows that the absorption band peak values are same at all frequency bands on both tannin extracts at 60°C and 90°C . The study also reveals that the greater absorption of peak band frequencies (Figure 9) of tannin extracted at 90°C . Thus, extraction of tannins from *A.xanthophloea* has greater efficiency at 90°C on aqueous medium than lower temperatures.

FTIR analysis was also used for study on the effect of different solvents that used for the extraction of tannins from *A.xanthophloea*, and the corresponding spectrum is given in Figure 10.

It shows the corresponding frequency band peak values like aqueous tannin extract at 90°C even in methanol and its mixture with water. But, the IR band peaks of methanolic extract is very sharp in all frequency range than others and the frequency bands of methanolic tannin extract of sample has greater absorption (shown in Figure 10) that confirms the tannins and other polyphenols that extracted well on methanol than water and mixture of solvents.

Figure 10. FTIR spectrum of *Acacia xanthophloea* tannins extract using different solvents.

4. CONCLUSIONS

The current research work spotlights the tannins extraction and characterization that obtained from *Acacia xanthophloea* bark. Experiments carried out and drawn the following conclusions:

Moisture content of the tannin sample estimated about $17.86 \pm 0.51\%$. Hence the sample does contain greater amount of phytochemical especially tannins.

Methanol, water and its mixture used as solvent, among these % yields of tannin methanolic extract has 52.55% . This is due to its being organic polar solvent.

UV-visible spectroscopy of sample was carried out, results confirms the availability of tannins and flavanoid ring system. Also, this study supporting to use the water as extracting solvent for tannins from *A.xanthophloea*.

FTIR study reveals that the characteristic absorption peak of polyphenols in all kinds of extracts. This study also revealed greater efficiency of tannins that extracted at 90°C by using water as solvent. Also, methanolic extract of sample has greater absorption bands among the other solvents used for extraction of tannins.

Thus, this study confirms and recommends that the methanol and water is to use as solvent for the extraction of tannins from *A.xanthophloea* like other acacia species. Most of characterization of the present study favors that methanol is more appropriate solvent than water.

5. RECOMMENDATIONS

Results of this study established and recommending using the easy accessible and eco-friendly tanning material could probably be achieved that are available around us. For those wishing to make their own tanning solutions obtained from extraction of tannins using suitable solvents and it is a less expensive method and probably the most common methods. Alcohol, water and its mixture could be recommended as usage for extracting the tannins, which is a simple common method and best suited for *A.xanthophloea* tannins like species. The extracted tannins could be applied on the skin/hides to process into finished leather

which might be advisable and useful as leather material for making of some leather goods.

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