Spectral characterization and HPLC profiling of a novel vegetable tanning agent extracted from the stem bark of an indigenous plant *Xylocarpus granatum*

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Abstract

Animal skins are tanned to convert into durable and flexible leather consisting of a multi-dimensional intimate structure. Although chromium tanned leather shows higher quality and strength properties, it is alarming and could be hazardous due to various environmental aspects. For this reason, vegetable extracts from natural sources have been prioritized for the tanning process as an alternative to chrome tanning. In this research, an initiative has been made to extract vegetable tannin from the *Xylocarpus granatum* bark using different solvents (e.g., water, methanol, ethanol, and chloroform) and a comparison was made with conventional tanning agent mimosa and quebracho tannin. Among all, extraction efficiency of methanol (100%) and ethanol (100%) were found higher than that of chloroform (100%) and water (100%) and the order was: methanol (31.22%) > ethanol (30.76%) > water (10.34%) > chloroform (6.22%). Chemical tests that include $FeCl_3$, gelatin, and $FeSO_4$ solution test of the extracted bark sample showed green-black, white, and violet color precipitate respectively which indicated the presence of tannin. The UV-visible and Fourier Transform Infrared spectroscopic investigations have demonstrated that *Xylocarpus granatum* bark contained tannins and flavonoids. High-Performance Liquid Chromatography (HPLC) investigation had revealed the presence of a higher amount of tannin content and polyphenolic compounds, e.g. epicatechin (503 mg/100 g dry extract), catechin hydrate (218 mg/100 g dry extract), catechol (29 mg/100 g dry extract). Moreover, condensed tannins, moisture content, and pH of the extracted bark were found 47.80%, 5.82%, and 3.82, respectively. Therefore, the extracted bark tannin could be a well alternative to the existing vegetable tanning materials.

Introduction

Raw animal hides and skins with a multi-dimensional intimate structure are tanned into durable and flexible leather. The production and transformation of raw hides and skins into leather involves several steps, including pre-tanning, tanning, and post-tanning [1]. Leather processing involves a variety of tanning procedures, materials, chemicals, and mechanical processes, with chrome tanning accounting for just 88-90% [2]. Vegetable tanning, aldehyde tanning, oil tanning, metal tanning (Zirconium, Titanium, Aluminium, Iron), and combination tanning are some of the other tanning procedures. Although chrome tanning shows better strength properties, only major concern is environmental pollution, as several studies and statistics show that only 54-57 percent of chromium is consumed during tanning, and the remaining chromium in tanning bath liquor is discharged after tanning, requiring special treatment before being released into the environment [3, 4]. Vegetable tanning, an alternative technique of leather processing have the most promising benefits since they are derived from plants and make leather that is full, compact, and embossable [3]. Mimosa, quebracho, sumac, tara, valonea, divi-divi, oak, and chestnut are the most often utilized chrome-free tannins nowadays.

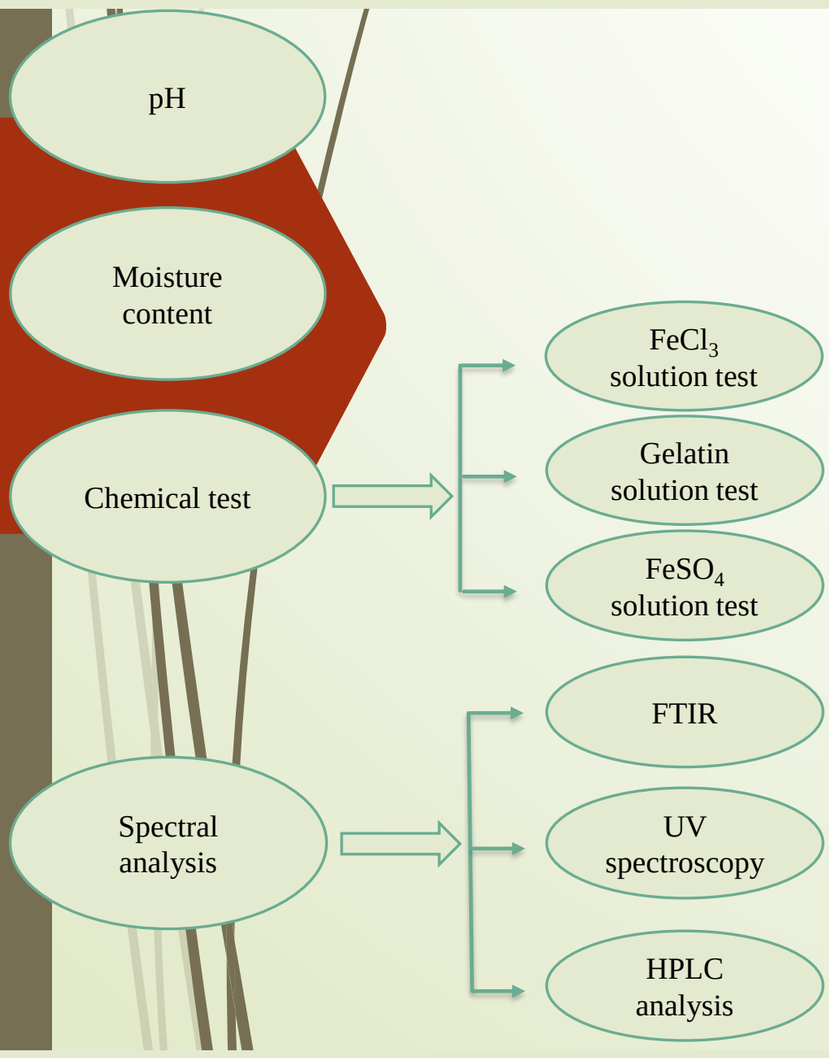
The bark of Xylocarpus granatum has never been used as a new source of vegetable tanning material. The goal of this study was to investigate–Xylocarpus granatum bark extract as a substitute for vegetable tanning agent.

Materials and Method

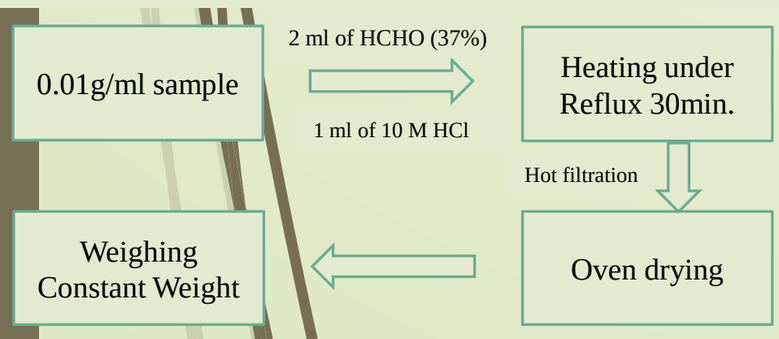
Xylocarpus granatum locally known as "Dhundul" bark sample was collected from the world's largest mangrove forest Sundarban, Khulna, Bangladesh.

Tannin extraction from bark

Characterization of extracted tannin



Condensed Tannin Analysis



Results and Discussion

Table-1: Extraction percentage of tannin by different solvents

Solvents	Initial weight of sample (g)	Weight after extraction (g)	Percentage of extract material
Water	40	4.14	10.34
Chloroform	40	2.48	6.22
Ethanol	40	12.31	30.76
Methanol	40	12.48	31.22

Table-2: Chemical tests for the presence of tannin in *Xylocarpus granatum* extracted sample

Reagents	Solvent for extraction	Observation	Inference (Tannin)
Ferric chloride Solution	Methanol	green-black precipitate	Present
	Ethanol	green-black precipitate	Present
Gelatin Solution	Methanol	white precipitate	Present
	Ethanol	white precipitate	Present
Ferrous sulfate Solution	Methanol	violet color precipitate	Present
	Ethanol	violet color precipitate	Present

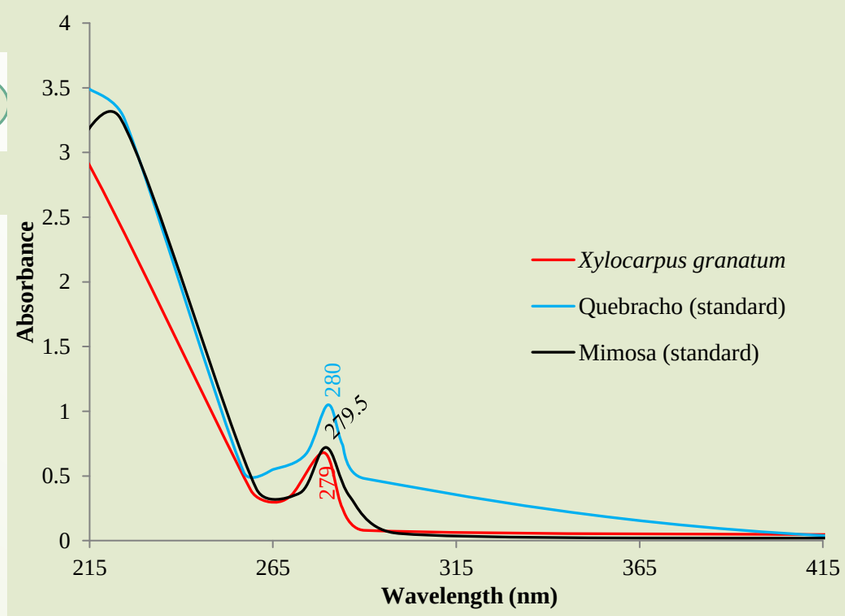


Figure-1: UV Spectra of *Xylocarpus granatum extract*, mimosa, and quebracho tannin

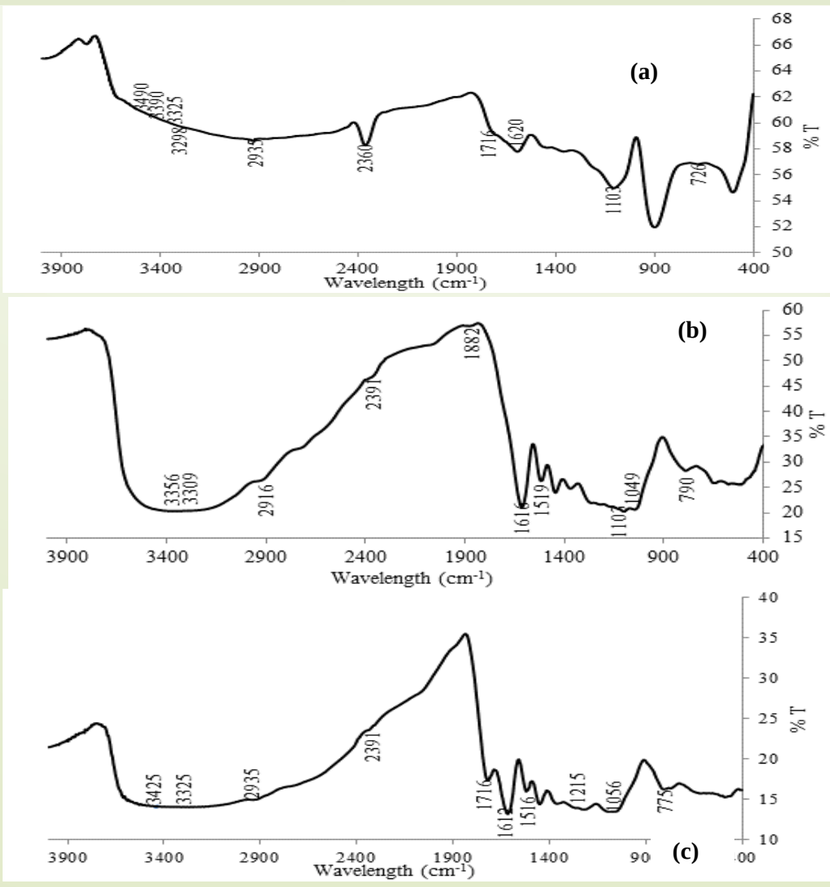


Figure-2: (a) FTIR spectra of standard mimosa tannin, (b) quebracho tannin, and (c) *Xylocarpus granatum* extracts

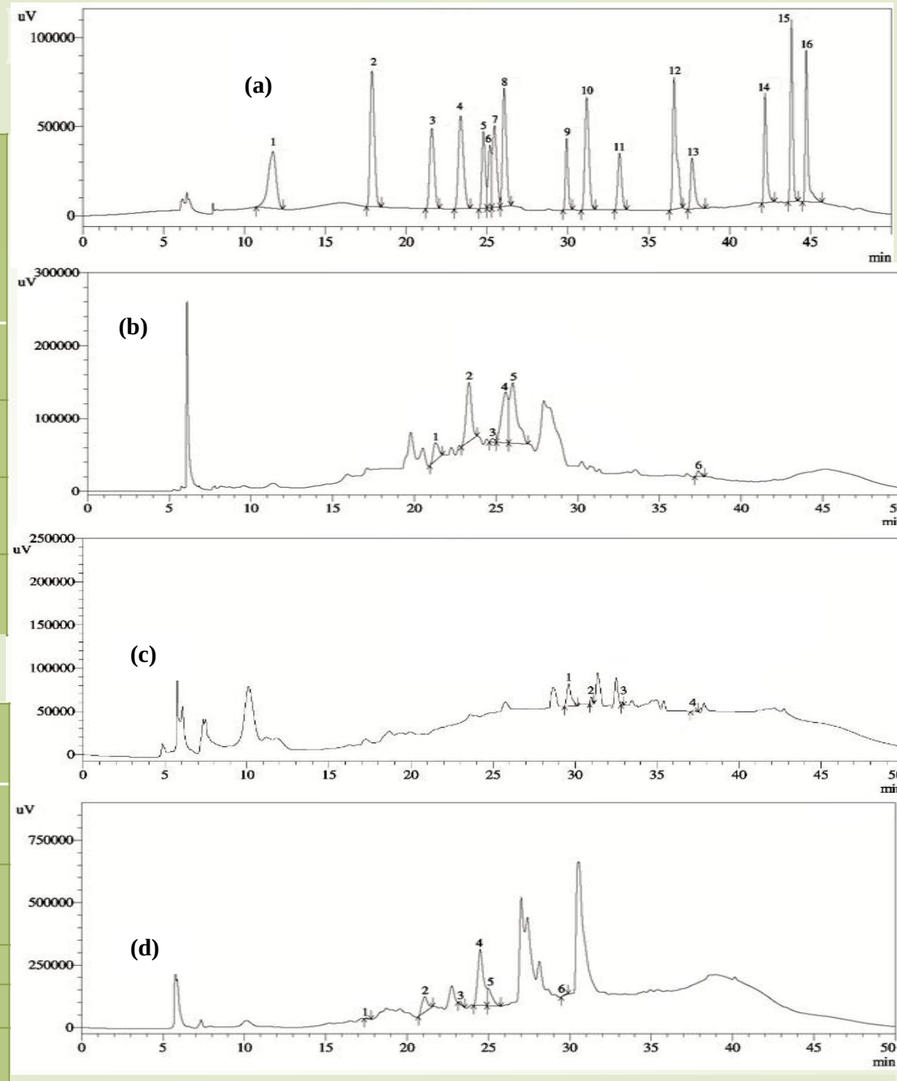


Figure-3: (a) HPLC chromatogram of a standard mixture of polyphenolic compounds, (b) mimosa tannin, (c) quebracho tannin, and (d) *Xylocarpus granatum* extract tannin

Conclusion

- **The maximum extraction efficiency of X. granatum bark tannin was found for methanol solvent (31.22%)**
- **The pH was found 3.82 for developed tannin which was closer to standard tannin (mimosa, quebracho) pH**
- ***Xylocarpus granatum* bark contains a significant quantity of condensed tannin and polyphenolic groups revealed by UV, FTIR, and HPLC analysis**
- **The chemical tests showed the presence of significant tannin content in the extract**

References

1. Hussein S.A. (2017) Utilization of tannins extract of acacia seyal bark (taleh) in tannage of leather. Journal of Chemical Engineering and Process Technology. 8: 334-342. doi: 10.4172/2157-7048.1000334
2. Mahdi H., Palmina K., Gurashi A.G., Covington D. (2009) Potential of vegetable tanning materials and basic aluminum sulphate in sudanese leather industry. Journal of Engineering Science and Technology. 4: 20-31
3. Adiguzel-Zengin A.C., Zengin G., Kilicarislan-Ozkan C., Dandar U., Kilic E. (2017) Characterization and application of acacia nilotica l. as an alternative vegetable tanning agent for leather processing. Fresenius Environmental Bulletin. 26(12): 7319-7326
4. Kuria A., Ombui J., Onyuka A., Sasia A., Kipyegon C., Kaimenyi P., Ngugi A. (2016) Quality evaluation of leathers produced by selected vegetable tanning materials from laikipia county, Kenya. IOSR Journal of Agriculture and Veterinary Science. 9(4): 13-17. doi: 10.9790/2380-0904011317
5. SLC-113 (1996) Determination of moisture content of natural ground materials. Official Methods of Analysis
6. SLC-120 (1996) Determination of pH. Official Methods of Analysis
7. Ahmed K.S., Jahan I.A., Jahan F., Hossain H. (2021) Antioxidant activities and simultaneous HPLC-DAD profiling of polyphenolic compounds from *Moringa oleifera* Lam. Leaves grown in Bangladesh. Food Research. 5(1): 401-408. doi.org/10.26656/fr.2017.5(1).410

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