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Research Article

Comparative Study of Traditional Decoction Method with the Conventional Ethanol extraction by Using Leaves and Barks of *Twerianudiflora*

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Abstract

The plant Trewia nudiflora is locally known as Lattu, Latim, Laddu tree in Bangladesh* and *has many reported pharmacological activities. The aqueous crude extract (decoction) is popularly used for the household treatment. In the present study, the extraction efficiency of the decoction method was compared to the conventional ethanolic extraction by quantitative and qualitative phytochemical analysis by using leaves and barks of T. nudiflora. Both fresh and dried parts of the plants were used for decoction to justify the importance of following the time-consuming drying process of plant materials traditionally practiced before any extraction. Though better extraction yield was observed in ethanolic extraction the presence of phytochemicals were almost similar to the decoction from dried leaves and barks. Whereas though the similar yield extraction were observed in decoction both from fresh and dried leaves and barks, comparatively less phytochemicals were present in the decoction obtained from fresh plant's parts. However, considering the extraction yields and the presence of phytochemicals proved the ethanolic extraction better than the decoction methods. Unfortunately crude extract obtained by decoction and ethanol from fresh and dried leaves and barks failed to show favorable antimicrobial activities E. coli made question marks on the pharmacological properties of that plant. However, further studies are required for getting a decision regarding antimicrobial properties of the plants.

Key words: *Trewia nudiflora*, Euphorbiaceae, decoction, phytochemicals, *E. coli*

1.Introduction

Trewia nudiflora Linn. belongs to the family of Euphorbiaceae (Ghai 2019) is locally known as Lattu, Latim, Laddu tree widely found in Bangladesh, India, Malaysia, China etc. (Ghai 2019, Chaity 2020, Dinerstein 1988). The plant is generally a fast growing, soft woody versatile dioecious tree which grows inside the semi-evergreen and wet tropical woods which is a medium-sized deciduous tree with soft stem (Figure 1). Leaves are green, ovate-cordate, 10-18 cm long and 6-13 cm broad, margin entire or serrate, apex sharp and oppositely arranged. Petiole is 2.5-8 centimeters long. The fruits of *T. nudiflora* are enormous, hard green, and dull, turn colored during maturing and the seeds are employed for propagation of the tree (Dinerstein, 1988).

Different parts of the plant have reported diverse pharmacological activity (Ghai 2019). Leaves have traditional uses for the treatment of wounds, swelling, and flatulence, excessive bile, sputum, dysentery etc (Nadkami, 2002, Rastogi 2004 & Kulju 2007).

Similarly, root has evidence of use in the treatment of stomachic, rheumatism, cancer notably leukemia and hepato-biliary diseases (Rathore 2007). The aqueous extract is generally utilized for several household treatments in different parts of Bangladesh. Decoction is the traditional extraction method for aqueous extraction from plant parts generally practiced by the folk medicinal practitioners. In this method the plant parts are simply boiled in the fresh (distilled) water to extract out the medicinal components in the water. The mixtures are then filtered and the solutions are ingested with sweetening agents or just applied on the body for the treatment purpose. This is a cheap method and any one can perform without sound knowledge on extraction. Though this method is widely used in household treatment, it has some limitations for dissolving hydrophobic or oily components such as volatile oil, lipid etc. present in the plant parts. Whereas the conventional extraction method which is conducted by ethanol is suitable for extracting both the hydrophobic and hydrophilic components from the plant parts. But the ethanolic extraction is comparatively expensive and hazardous procedure and requires sound knowledge on extraction. Decoction method may be possible from both fresh and dried plants parts popularized this extraction method in traditional or household medicinal practitioners. On the other hand, like other conventional extraction methods, ethanolic extraction method is generally conducted by using the dried plant parts which is a time-consuming process and responsible for delaying the overall extraction process along with the cost.

The present study was primarily designed to compare the conventional ethanolic extraction method to the traditional decoction method on the basis of the extraction efficiency (quantitative properties) and the efficacy (qualitative properties) of the extract obtained from leaves and barks of *T. nudiflora*. Similarly, to explain the drying process of plant materials before extraction and the usefulness of this approach for medicinal purposes can also be compared by using both fresh and dried plant's parts (leaves and barks) of *T. nudiflora* in the decoction method.

Figure 1: Leaves and flower *Trewia nudiflora*

Figure 2: Fruits of *T. nudiflora*

2. Materials and Methods

2.1 Study protocol

The present study was conducted to compare the conventional ethanolic extraction method with the traditional decoction method by using the leaves and barks of *T. nudiflora*. Both fresh and shade dried plant materials (Table-1) were used in the present study (Cheenickal 2017) and comparisons were made by considering the extraction efficiency (quantity of the extract) and efficacy (quality of the extract). Extraction efficiency was firstly measured by comparing the yield value of the crude extract as per Equation-1. Extraction efficiency was also assessed by comparing the presence of different types of phytochemicals (Table 2). Extraction efficacy (pharmacological properties of the extracts) were assessed by using antimicrobial sensitivity study by using *E. coli* bacteria.

2.1 Accumulation of specimen

Both leaves and barks of *Trewia nudiflora* were collected from Shahjadpur of Sirajganj District of Bangladesh in November 2021. The plant was previously identified by an expert and a herbarium sheet was preserved in the department for further reference.

2.2 Extraction Procedure

Fresh leaves and barks were washed properly by running tap water followed by distilled water and allowed for shade drying to remove the surface water which was further divided into three parts (Part A, B and C), 100 gm in each. The leaves of Part-A were allowed for decoction immediately after dividing, whereas Part-B and Part-C of fresh leaves were allowed for weeklong drying. After grinding dried leaves of Part-B and Part-C were allowed for decoction and ethanolic extraction respectively (Table-1). The above procedure was also performed for the bark of *Trewia nudiflora* described in Table-1. The extracted specimen was filtered through a cotton plug followed by Whatman No.1 filter paper with lower pressure and finally rotary evaporation (in vacuum at 40^0C) was used to concentrate. The dried crude extracts were then placed in an airtight container labeled as (i) **EL-1** (crude extract obtained by decoction of fresh leaves of *T. nudiflora*), (ii) **EL-2** (crude extract obtained by decoction of dried leaves powder of *T. nudiflora*), (iii) **EL-3** (crude extract obtained by ethanolic extraction of dried leaves powder of *T. nudiflora*), and (vi) **EL-4** (crude extract obtained by decoction of fresh barks of *T. nudiflora*), (v) **EL-5** (crude extract obtained by decoction of dried barks powder of *T. nudiflora*), (vi) **EL-6** (crude extract obtained by ethanolic extraction of dried barks powder of *T. nudiflora*). Extraction yield calculations (Table-2) were performed immediately after drying the crude extracts whereas phytochemical screening test (Table-3) and antimicrobial study were performed as per suitable time by conforming standard laboratory setup and procedure. All procedures were repeated three times and the average result was counted for statistical analysis.

Table 1: Extraction procedure of *Trewia nudiflora* leaves and barks

Method	Plant parts	Method of extraction	Description of the extraction procedure
A	(I) Fresh leaves (II) Fresh barks	Decoction	Plant parts were blended in a conventional blender machine to prepare a juice and diluted to 500ml by adding distilled water. The obtained juice was boiled for 5 minutes before extraction (Sadat, 2021)
B	(I) Powder of dried leaves (II) Powder of dried barks	Decoction	Fine powder obtained from dried leaves and barks were separately mixed with distilled water in a 1:5 ratio and allowed for boiling 5 minutes before extraction (Sadat, 2021).
C	(I) Powder of dried leaves (II) Powder of dried barks	Ethanolic extraction	Fine powder obtained from dried leaves and barks were separately mixed with ethanol in a 1:5 ratio and allowed for cold extraction up to 72 hours with intermittent shaking as per standard method (Latha, 2015).

Here, comparison between "Part-A" and "Part-B", and "Part-B" and "Part-C" will be considered in respect to efficiency (extraction performance such as amount of crude extract obtained from the extraction method and quantity of the phytochemicals present) and efficacy (pharmacology properties).

2.3 Percentage of Yield Calculation:

The percentage of yield (Table-3) indicates the efficiency of the extraction procedure which was calculated by using the following formula (Terblanche, 2017; Sadat, 2018).

$$\%\, Yield = \frac{(W1\ x\ 100)}{W2} \text{ ---------- Eq. 1}$$

Here, $W1$: weight of dried crude extract

$W2$: weight of the starting plant material for extraction

Table 2: Phytochemical Screening Test of *Trewia nudiflora*

Phytochemicals	Qualitative test
Alkaloids	The presence of alkaloids in the sample may be determined either by (i) Dragendoff's test (an orange red precipitate was obtained by heating the sample mixed with 2% of H_2SO_4 and few drops Dragendoff's reagent) (Trease,1989) or (ii) Mayer's test (a turbidity or yellow precipitation were observed by heating the sample mixed with 2% HCl and few drops Mayer's reagent) (Dash, 2017).]
Anthraquinones	The presence of anthraquinones in the sample were determined by changing the sample color (pinkish) after adding the benzene or chloroform with 10% (v/v) ammonia solution in the sample (Ajayi, 2015; Ayoola, 2008)
Flavonoids	The presence of flavonoids in the sample were determined either by (i) adding dilute ammonia solution with Conc. H_2SO_4 in the sample gives an indicative yellow coloration that disappears on standing (Ayoola, 2008) or (ii) just adding a few drops of 1% aluminum solution in the sample turns the yellow coloration (Ayoola, 2008).
Glycosides	The presence of glycosides in the sample may be determined by adding 3 ml of glacial acetic acid with 1 drop of 5% ferric chloride Solution and 0.5 ml of Conc. H_2SO_4. An indicative brown or blue ring may be observed in the interface of the mixture (Ayoola, 2008).
Saponins	After vigorous shaking of the sample mixed with water, creating foam stable for more than 10 minutes indicates the presence of saponins in the sample (Dash, 2017).
Steroids	The presence of steroids in the sample may be determined by adding 2 ml acetic anhydride and 3 ml of Conc. H_2SO_4 in the sample solution creates a layer. An indicative color change from violet to blue was observed (Ayoola, 2008).
Terpenoids	The presence of terpenoids may be determined by adding 2 ml Chloroform with 3 ml Conc. H_2SO_4 in the sample solution creates a layer. An indicative reddish-brown color may be observed in the interface (This is known as Salkowski test) (Ayoola, 2008).
Tannins	The presence of tannins may be determined by observing dark green color of the sample solution after adding 1% $FeCl_3$ solution (Maxson, 1972)
Anthraquinone	The presence of anthraquinone may be determined by adding 5 ml of $CHCl_3$ and 10% ammonia solution in the sample solution. An indicative bright pink color may be observed in the aqueous layer (Hazali, 2015).
Vitamin C	The presence of Vitamin C may be determined by adding 1 drop of 5% w/v sodium nitroprusside, 1 ml of diluted NaOH and 0.4 ml of HCl in the sample solution that turns the yellow color to blue (Hazali, 2015).
Phenol	The presence of phenol may be determined by observing blackish green coloration of the sample solution after adding 2 ml of alcohol and few drops of ferric chloride solution (Hazali, 2015).

1. Antimicrobial Study

Disc diffusion method (Baker 1993, Mukhtar 2000, Servan 2007; Abedin 2021) is a popular method for antimicrobial sensitivity study. In the present study Gram negative bacteria *Escherichia coli* was used for measuring the antimicrobial properties of the crude extracts obtained from *T. nudiflora* leaves and barks applying different extraction procedures in the present study. The microorganism was collected from the Microbiology Lab, Department of Microbiology, Khwaja Yunus Ali University, Bangladesh. Using a pair of sterilized forceps, the 400g/disc of extract-impregnated filter paper discs were put on the surface of the infected nutritional agar medium. After thirty minutes of pre-diffusion, the Petri dish was put in an

incubator at 37^0C for twenty-four hours. The sensitivity of the organisms to the crude extracts were assessed by measuring the width of visible inhibitory zones to the closest millimeter. The procedure was repeated three times and the average result was counted for statistical analysis.

2. Results and Discussion

In the present study extraction efficiency was compared between aqueous decoction and ethanolic extraction method obtained from *Trewianudiflora*. The overall ethanolic extraction both from leaves and bark of *T. nudiflora* were observed higher than the decoction method (Chart 1). It was also observed that the decoction method did not show any privilege by using the fresh or dried plant's parts ($p>0.05$) for extraction. Ethanolic extraction was significantly higher than both forms of decoction method involved in barks, where the difference was not significant in case of decoction applied on dried leaves (Table 3). Highest extraction was observed in leaves extracted by ethanol. Based on the extraction yield it was observed that conventional ethanolic extraction showed better extraction efficiency than the decoction method traditionally used in household practice. However, phytochemical screening tests indicates that decoction method (Figure-3) from dried leaves successfully capable of extracting out most of the phytochemicals such as the tannins, flavonoids, saponins, phenols, glycosides, alkaloids and vitamin C which was almost similar to the ethanolic (Figure-4) extract from dried leaves (Table 4). Not only that, during the phytochemical studies it was observed that the decoction method successfully extracted vitamin C from the leaves whereas ethanolic extract failed to do so (Figure 3). Ripa, (2022) observed similar composition on methanolic leaf extract of *T. nudiflora* which was also present after ethanolic fraction after TLC. In the present study, extraction from the barks also showed almost similar composition of compounds in the dried extracts obtained by the decoction (Figure-5) and ethanolic method (Figure-6). However, ethanolic extract additionally extracted the terpenoids from the bark (Table-4). From the above study it was observed that the decoction method was suitable for dried leaves whereas ethanolic extraction method for dried barks powder. Crude extracts obtained by decoction from fresh leaves and barks were observed to have poor phytochemical composition (Table-4). Though the crude extract from leaves and bark of *T. nudiflora* were observed rich in different phytochemicals but failed to show favorable antimicrobial activities on *E. coli* (Figure 6) indicated its lack of antimicrobial properties. However, Sultana (2022) observed potential antibacterial activity of methanolic extract of *T. nudiflora* against several Gram positive such as *S. gallinarum, S. sciuri, S. iniae, S. constellatus* etc. and Gram -ve bacteria such as *A. cavernicala, A. diversa, E. anguillarum, E. xiangfangensis, S. colletis, V. rotiferianus, X. axonopodies, X. campestris,* etc. So, further antimicrobial studies are required to get a final decision regarding antimicrobial properties of ethanolic extract of *T. nudiflora*.

Table 3: Yield variation of different extraction methods

Plant materials	Method of Extraction	Starting materials (gm)	Weight (gm) after drying (Mean±SEM)	Solvent used (ml)	% Yield	p (Yield variation)
Leaves	A(I)*	50	-	q.s. to 500	3.31±1.27	-
	B(I)*	50	19.33±1.52	5 times to the dry wt	4.87±0.99	0.364^x
	C(I)*	50	$18.67±2.082^a$	5 times to the dry wt	9.41±2.77	0.043^x 0.172^y
Barks	A(II)*	50	-	q.s. to 500	1.18±0.52	-
	B(II)*	50	35.67±1.53	5 times to the dry wt	3.56±1.82	0.073^x
	C(II)*	50	$36.33±2.52^a$	5 times to the dry wt	4.47±1.052	0.011^x 0.022^y

* Method of extraction as per Table-1. Mean calculated by considering successive 3 studies.

[a] Significance level compared with "B", where $p \geq 0.05$, is statistically insignificant at 5% level of significance.
[x] Significance level compare with "A", where $p \leq 0.05$, is statistically significant at 5% level of significance.
[y] Significance level compared with "B", where $p \leq 0.05$, is statistically significant at 5% level of significance.

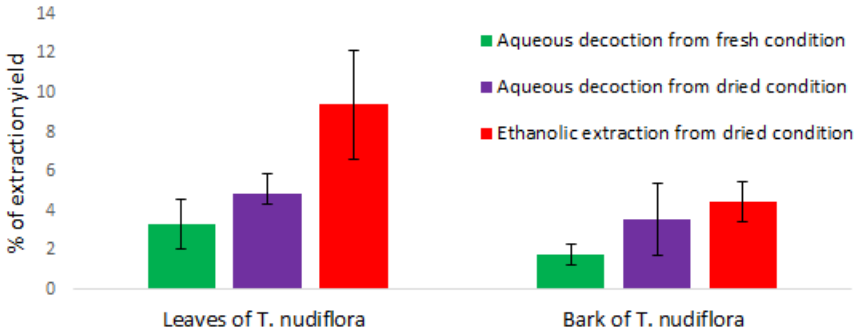

Chart 1: Extraction yield of different parts of *T. nudiflora*in different extraction method

Table 4: Qualitative studies of phytochemicals present in the crude extracts

Chemical Compounds	Tests/Reagent	Aqueous decoction from fresh Plant parts		Aqueous decoction from dried plant parts		Ethanolic Extraction from dried plant parts	
		Fresh Leaf juice	Fresh Bark juice	Dried Leaf powder	Dried Bark powder	Dried Leaf powder	Dried Bark powder
Tannins	Ferric Chloride	+	-	+	-	+	-
	Lead Acetate	+	+	+	-	+	+
Flavonoids	Sulfuric Acid	+	+	+	+	+	+
	Aluminum	+	+	+	+	+	+
Saponins	Foam Test	+	+	+	+	+	+
Steroids	Liebermann-Burchard Test	-	-	-	-	-	-
Terpenoids	Liebermann-Burchard Test	-	-	-	-	-	+
Glycosides	Keller's reagent	-	-	+	+	+	+
Alkaloids	Dragendorff's Test	-	-	+	+	+	+
	Mayer's Test	-	-	+	-	+	+
Anthraquinones		-	-	-	-	-	-
Vitamin C		-	-	+	-	-	-
Phenols		+	+	+	+	+	+

Note: "+" indicates presence, whereas "-" indicates absence of the respective compounds.

(a) (b) (c) (d)

Figure 3: Evidence of presence of (a) tannins, (b) flavonoids, (c) saponins, (d) phenols in the crude extract of aqueous decoction of fresh leaves

(a) (b) (c) (d) (e) (f)

Figure 4: Evidence of presence of (a) tannins, (b) flavonoids, (c) saponins, (d) glycoside, (e) alkaloid (f) phenols in the crude extract of ethanolic leaves extracts

(a) (b) (c) (d)

Figure 5: Evidence of presence of (a) tannins, (b) flavonoids, (c) saponins, (d) phenols in the crude extract of bark obtained by decoction

(a) (b) (c) (d) (e) (f) (g)

Figure 6: Evidence of presence of (a) tannins, (b) flavonoids, (c) saponins, (d) terpinoid (e) glycoside, (f) alkaloid (g) phenols in the crude extract of ethanolic bark extracts

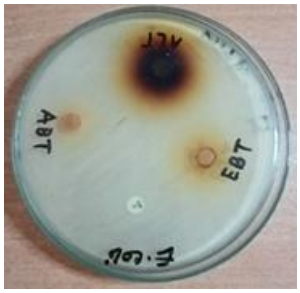

Figure 7: Antimicrobial study filed to create zone of inhibition

3. Conclusion

Trewia nudiflora was phytochemically a rich plant and available in our surroundings. Satisfactory extraction was found from dried parts of the plant by using both decoction (traditional extraction method) and ethanolic (conventional extraction) method. Decoction was proved better to extract out water soluble components such as Vitamin C from the leaves powder whereas ethanol successfully extracted the terpenoids like lipophilic components from the barks. Unfortunately, the extracts obtained from decoction or ethanol proved unfavorable antimicrobial activities against *E. coli* (gram –ve) bacteria which was not supportive to the previously published similar type of studies (Sultana, 2022). Further antimicrobial study is required on other reference microorganisms before getting any concrete decision regarding the antimicrobial properties of the crude extract of the *T. nudiflora*.

6. Conflict of interest

The authors declare that they have no conflict of interest.

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