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# Extraction of tannin by *Terminalia bellirica* (Gaertner) roxb seed from different provenances

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#### **Abstract**

Bahera (*Terminalia bellirica*) (Gaertner) Roxb) seeds contain a high amount of tannins such as cheblagic acid, tannic acid and gallic acid belong the hydrolysable group and are extensively used for medicinal purposes. It is used as antioxidant, antimicrobial, antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent. This study was carried out to compare tannin content in seeds of Terminalia bellirica from five different provenances (Raipur, Bilaspur, Jagdalpur, Kawardha and Raigarh) of Chhattisgarh. The results reveals that maximum tannin content was in seeds from Raipur provenance, minimum was in seeds from Raigarh. So, we concluded that the quantitative and qualitative variation in tannin content of seeds of five provenances was due to their genotypes as there is variation in the climatic condition of these regions.

Keywords: Terminalia bellirica, Tannins, Seed extract, Phytoconstituents, Provenances,

## INTRODUCTION

Tannins are astringent, bitter-tasting plant polyphenols that bind and precipitate proteins, applied to any large polyphenolic compound containing sufficient hydroxyls and other suitable groups (such as carboxyls) to form strong complexes with proteins and other macromolecules<sup>1</sup>. They are naturally occurring, high molecular weights (ranging from 500 to 3000) polyphenolics found in higher plants including many plants used as foods and feed2. Tannins ingested with the diet by humans or animals may affect protein utilization by forming insoluble complexes with protein, iron utilization by complexing with iron, and biological antioxidant status by participating in redox reactions3. Tannins may contribute to the chemical defenses that minimize damage to plants by insect and mammalian herbivores4. Their importance in nutrition and in ecological processes makes qualitative and quantitative analysis of tannins critical to studies of plant nutritional quality and plant ecology<sup>5</sup>. Constraints on effective chemical analysis include the variable composition of tannins within plants, which includes variation with species, season, and environmental stresses such as herbivory<sup>6</sup>. It constitutes a very diverse group of oligomers and polymers. They are classified into two broad groups, namely hydrolysable and condensed. Condensed tannins however are more resistant than hydrolysable tannins7. Tannins such as chebulagic acid, tannic acid and gallic acid belong to hydrolysable group and are extensively used for medicinal purposes8,9. Gallic acid may be employed medicinally in analgesic, antiallergenic, antibronchitic, antiinflammatory, antioxidant, antiperoxidant, antiviral, bacteristat, bronchodilator, immunosuppressant, immunosuppressant, and

phytoconstituents like Gallo-tannic acid, bellericanin, ellagic acid, gallic acid, termilignan, flavone and anolignan B, Tannins, chebulaginic acid, phenyllemblin, sitosterol, mannitol, glucose, fructose and rhamnose. These compounds are responsible for many activities such as antioxidant, antimicrobial, antidiarrheal, anticancer, antidiabetic, antihypertensive and hepatoprotective agent<sup>12</sup>. In Ayurveda the drug is classified as an expectorant. It is an integral part of Ayurvedic laxative formulation, Triphala used in treatment of common cold, pharyngitis and constipation<sup>14,15,16,17,18</sup>. The bark is

diuretic, useful in anaemia and leucoderma. Fruits are astringent,

acrid, digestive, anthelmintic, aperient, expectorant, sweet, anodyne,

stypic, narcotic, ophthalmic, antipyretic, antiemetic and rejuvenating.

Unripe fruit is a mild laxative and ripe fruit is an astringent. Seeds are

used as aphrodisiac. Oil extract from the seed pulp is used in

astringent<sup>10</sup>. In view of this our attention has been focused

particularly on Terminalia belerica belongs to family Combretaceace

deciduous tree with bluish or ashy - grey bark and about 20- 25 m

height<sup>12</sup>. It is found in abundance in Madhya Pradesh, Uttar Pradesh,

Punjab, Maharashtra states of India and also in Sri Lanka and

Malaya. It also occurs in different parts of India excepting the dry

region of Western India<sup>13</sup>. It contain many

Terminalia belirica commonly known as Bahera. It is a large

essential

which is a rich source of tannins<sup>11</sup>.

leucoderma and alopecia<sup>5,9</sup>.

Crude methanolic extract of the fruits of *Terminalia belerica* Roxb along with its various organic fractions elicited both *in vitro* and *in vivo* antioxidant activity as well as antibacterial activity<sup>19</sup>. *T. belerica* fruit rind powder was assessed for its antimicrobial activity by using Chloroform-Ethyl Acetate fractions. The fractionized compound Epigallo catechin gallate showed significant antimicrobial activity against *E.coli*, *B. subtilis and S.Aureus*<sup>20</sup>. The Anti diarrhoeal activity was performed using Castor oil induced diarrhoea, PGE2 induced entero pooling and gastrointestinal motility test<sup>21</sup>. Latha et al<sup>22</sup> investigated antidiabetic activity of *T. belerica* fruit extracts at the doses of 200, 300 and 400 mg/kg, p.o for 60 days to Streptozotocin induced diabetic rats significantly (p<0.05) increased the plasma insulin, C-peptide and glucose tolerance levels, body weight, serum total protein. Shukla et al<sup>23</sup> were evaluated the

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protective effect of TB fruit extract and its active principle, Gallic acid against CCl4 intoxication. Therefore, the present study was designed to extract the tannin from the seeds of the *Terminalia bellirica*.

# MATERIAL AND METHOD Collection of plant material

Seeds of five different provenances were collected from Raipur, Bilaspur, Raigarh, Jagdalpur and Kawardha. The seeds were dried under shade and kept them in a dry place in the dark.

# Extraction of tannins Solvent extraction process

Dried and ground seeds (10 gm *Terminalia* seeds) of each provenances were extracted with 500 ml of 50% aqueous methanol for 24 h in Soxhlet separately. Each extracts were evaporated on a water bath and filtered to remove fats and then freeze-dried. This extract was used as sample for tannin analysis<sup>24</sup>.

# **Analysis**

The detection of tannins was done by thin layer chromatography (TLC). TLC was performed on 0.25 mm thick silica gel layer using methanol: chloroform (2:1 v/v) as a solvent system, equal volume (2  $\mu$ I) of the extract from each of the samples was spotted on the TLC plate and compared with the standard tannic acid. After air-drying for 20 min, the presence of tannic acid was visualized by spraying the plate with 0.1% (w/v) FeCl<sub>3</sub> reagent<sup>24</sup>.

# Calculation of R<sub>f</sub> value

The movement of samples was characterized by a retardation factor  $(R_{\mbox{\scriptsize f}})$  defined as follows:

R<sub>f</sub> = Distance moved by the analyte from the origin

Distance moved by the mobile phase front from the origin

# Estimation of Tannins by spectrophotometry method

The quantification of tannins was made by spectrophotometry<sup>25</sup>. Standard solutions reagents were prepared for Folin Denis method.

# Folin-Denis reagent

To 750 ml of water, 100 g of sodium tungstate (Na<sub>2</sub>WO4.2H2O), 20 g of phosphomolybdic acid and 50 ml of 85% phosphoric acid ( $H_3PO_4$ ). Reflex mixture for 2 hr, cool to 25 °C and dilute to 1000 ml with water.

#### Saturated sodium carbonate solution

To 100 ml of water, add 35 g of anhydrous sodium carbonate, dissolve at 70-80  $\,^{\circ}\text{C}$  and cool overnight. Decant the clear liquid before the use.

#### Tannic acid standard solution

Dissolve 100 mg of tannic acid in 1 litre of water. Prepare

fresh solution for each determination (1 ml = 0.1 mg of tannic acid).

# Preparation of sample

Dissolve 1 g of sample with 80 ml of water, transfer to 100 ml volumetric flask and dilute mark. Shake well and filter.

## Determination

Use an aliquot of the filtrate containing not more than 0.1 mg of tannic acid (Table 2).

# RESULT AND DISCUSSION Thin layer chromatography

The identification of tannins was made by thin layer chromatography (TLC). 2  $\mu$ I (0.1 mg/ml in methanol) of the extract from each five samples was spotted on the TLC plate and compared with the standard tannic acid. After air-drying for 20 min, the presence of tannic acid was viewed by spraying the plate with 0.1% (w/v) FeCl<sub>3</sub> reagent. The movement of tannin in standard and seed samples was characterized by a retardation factor (R<sub>f</sub>). The R<sub>f</sub> values of standard tannic acid was 0.9 cm, seed sample of Raipur provenance 0.9 cm, Kawardha provenance 0.9 cm, Bilaspur provenance 0.9 cm, Raigarh provenance 0.85 cm and Jagdalpur provenance 0.85 cm (Table 1, Fig 1). The observation showed the presence of tannic acid in seed samples of all five provenances.

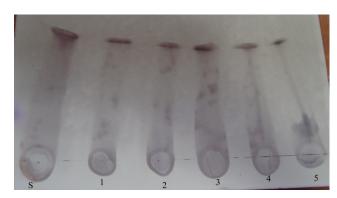


Fig 1. TLC of tannin: Lane s shows standard sample and lanes 1-5 show samples from five provenances: 1-Raipur, 2-Kawardha, 3- Bilaspur, 4-Raigarh and 5- Jagdalpur.

### Quantification of tannin content

The quantification of tannins was made spectrophotometer. Standard solutions were formulated by Folin Denis reagent. Five standard tubes are made containing 0.1 mgl-1 tannic acid standard solution, tube number (T1 to T5) and five sample tube are made for five different places U1 for Raipur, U2 for Kawardha, U3 for Bilaspur, U4 for Raigarh and U5 for Jagdhalpur. The calibration curve was developed at 725 nm. The quantity of tannin in seed samples of five provenances were as follows: 136 µgml-1 in Raipur seed sample, 125 µgml-1 in Kawardha seed sample, 120 µgml-1 in Bilaspur seed sample, 113 µgml-1 in Raigarh seed sample and 105 µgml-1 in Jagdalpur seed sample (Table 2). It is concluded that amount of tannin is different in all five different provenances.

# Weight of tannin

The weight of tannin extracted in 10 gram seeds of each of the five provenances were as follows: Raipur seeds 25 mg, Kawardha seeds 20 mg, Bilaspur seeds 16 mg, Raigarh seeds 14 mg and Jagdalpur seeds 14 mg (Table 3).

Results indicated that the quantitative variation in tannin content of seeds of five provenances is due to their respective genotypes as there is variation in climate of these regions. Similarly, the qualitative variation in Rf values also appears to be genotypic variation. Phale and Madibela<sup>26</sup> reported amounts of soluble condensed tannins and neutral detergent fibre-bound tannins in fodder trees and forage crops in Botswana. They found variation in the amount of soluble tannin in different plant species. Robert and Burns<sup>27</sup> reported method for estimation of tannin in Grain Sorghum. Tabasum et al<sup>28</sup> analyzed tannin content in different food products. The products having least tannin contents were: Supreme Brooke Bond tea (0.18%), green tea (0.18%), Maxwell coffee (0.35%), Golden apple (0.19%), Ammri apple (0.19%), Naakh pear (0.02%), Surmai peach (0.09%) and Chalari pomegranate (0.39%). Suzana et al<sup>29</sup> reported the seasonal variation in the content of tannins in barks of barbatimao species Stryphnodendron adstringens (Mart.) Cov. and S. polyphyllum (Mart.). They analyzed the contents of tannins in the barks monthly during one year and correlated the results with climate conditions (rainfall, humidity and mean temperature) by chemometric methods. They found season-dependent variation in production of tannins. Lege et al30 reported condensed tannins reportedly act as feeding deterrents to bollwonn [Helicoverpa zea (Boddie)], tobacco budworm [Heliothis virescens (F.)], and two spotted spider mite [Tetranychus urticae (Koch)] in cotton [Gossypium hirsutum L.]. Condensed tannin concentration, expressed as a percentage of fresh weight, was determined spectrophotometrically following extraction with HC1-butanol. Hagerman (1980)<sup>31</sup> reported the extractability of tannin from fresh, lyophilized, and dried leaves collected at various times in the growing season was determined using the radial diffusion assay for proteinprecipitating phenolics. Kun et al<sup>32</sup> studied that tannin extracts from dried immature fruits of Terminalia chebula Fructus Retz. can promote cutaneous wound healing in rats and suggested that tannins are powerful anti-bacterial and angiogenic activity. Andrew and Barber<sup>33</sup> analyzed tannin content in Apple, pear, peach and pomegranate.

Table 1. Thin Layer Chromatographic separation of tannin extracted from seeds of five provenances

S.No.	Provenances	Distance moved by the analyte from the origin	Distance moved by the mobile phase front from the origin	R <sub>f</sub>
1.	Standard	9	10	0.9
2.	Raipur	9	10	0.9
3.	Kawardha	9	10	0.9
4.	Bilaspur	9	10	0.9
5.	Raigarh	8.5	10	0.85
6.	Jagdalpur	8.5	10	0.85

Table 2. Preparation of calibration curve for tannin

Tube no.	Tannic acid solution (0.1 mg <sup>-1</sup> )	Distilled water (ml)	Folin reagent (ml)	Sodium carbonate solution (ml)	Absorbance at 725nm	Tannic acid (µ g)	Tannic acid µg/ml
Blank		0.00	0.25	1.26	0.00	0	0
T1.		0.50	0.25	1.25	0.112	2	100
T2.		0.48	0.25	1.25	0.218	4	100
T3.		0.46	0.25	1.25	0.327	6	100
T4.		0.44	0.25	1.25	0.432	8	100
T5.		0.40	0.25	1.25	0.538	10	100
U1.		0.46	0.25	1.25	0.450	8.2	136
U2.	0.06	0.46	0.25	1.25	0.412	7.5	125
U3.		0.46	0.25	1.25	0.400	7.3	120
U4.		0.46	0.25	1.25	0.387	6.8	113
U5.		0.46	0.25	1.25	0.345	6.3	105

Table 3. Weight of Tannin

S No.	Provenances	Amount of seed powder (g)	Weight of extract (mg)	Tannin per gram seed (mg)
1.	Raipur	10	25.0	2.5
2.	Kawardha	10	20.0	2.0
3.	Bilaspur	10	16.0	1.6
4.	Raigarh	10	14.0	1.4
5.	Jagdalpur	10	14.0	1.4

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# **CONCLUSION**

Terminalia bellirica Retz. is an important medicinal tree which contains hydrolysable type of tannins Chen et al<sup>8</sup>. Maximum amount of tannin is present in the seeds of *T. bellirica*, popularly known as myrobalan. Tannins such as chebulagic acid, chebulinic acid, tannic acid and gallic acid belongs the hydrolysable group and are extensively used for medicinal purposes. This study shows that the amount of tannin obtained from Raipur provenance was 2.5 mg per gram of seeds, which is higher than Kawardha provenance (2.0 mg per gram of seeds) (Table 3). Seeds of Bilaspur provenance contained tannin 1.6 mg per gram seeds, and Lowest tannin was obtained from seeds of Jagdalpur and Raigarh provenance (1.4 mg tannin per gram of seeds). We are concluded that the quantitative and qualitative variation in tannin content of seeds of five provenances was due to their genotypes.

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