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Phytochemical And Elemental Analysis Of Stem Bark Of *Terminalia Bellerica* (Gaertn.)Roxb.

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Abstract: Plants are the primary source of several phytochemicals and mineral elements, these plays very important role in several biochemical processes in plants, animals and human beings. *T.bellerica* is an important ethnomedicinal plant known for curing several disorders of human beings. The medicinal properties of every plant may be due to the presence of several mineral elements like Ca, Cr, Cu, Fe, Mg, K and Zn. The detection of phytochemicals and mineral elements in plants is very much necessary to know the efficacy and quality of drugs. So the Present study was carried out to know the phytochemical constituents and concentration of various elements present in the stem bark of *Terminalia bellerica* by using standard procedurs and Atomic absorption spectrometer respectively. The qualitative study reveals the presence of Alkaloids, flavanoids, phenol, tannins, terpinoids, glycosides, saponins. The quantitative estimation study reveals the presence of high quantity of glycosides (1.86mg/g), flavanoids (1.28mg/g), phenols (1.24mg/g), alkaloids (1.20mg/g) and terpenoids (1.12mg/g) and the least quantity of steroids(0.84mg/g), saponins (0.58mg/g) and tannins (0.38mg/g) were observed. Similarly it also revealed the presence of fourteen elements like Calcium, Pottassium, Magnesium, Iron, Copper, Zinc, Manganese, Chromium, Aluminium, Cadmium, Silicon, Molybdenum, Titanium and Vanadium. Among the analysed elements, high concentration that is 30.0578mg/L of Silicon and 14.1266mg/L of Calcium were recorded. As for my knowledge this is the first report on elemental analysis of stem bark of *T.bellerica* and very few reports available on phytochemical studies on stem bark of this plant.

Index Terms-Atomic absorption spectrometer, Silicon, Stem bark, *Terminalia bellerica.*

I. INTRODUCTION

Plants are the magical creations of god, without plants life is unimaginable. All the organisms need food, shelter and medicine for their survival. Medicinal plants possesses bioactive molecules helps in resolving health issues of all living beings. Phytochemicals are the biproducts produced from plants during metabolic process and helps in protecting plants from diseases. Several mineral elements are involved in the biochemical process taking place in the plants, animals and human beings. The important sources of these elements for plants are rainfall, atmospheric dusts, plant protection agents and fertilizers that can be absorbed through the leaf blades (Lozak et al., 2002). The plants supplimented with optimum level of mineral elements, helps in the healthier growth and results in the production of potential phytochemicals of pharmaceutical importance. Mineral elements are not synthesized in the human body, but they are supplimented in their diet transferred from plants through their roots (Lozak et al., 2002). The Human body needs a number of mineral elements in order to maintain good health (Ajasa et al., 2004; Balaji et al., 2000). More than 40 elements have been considered essential to life systems for the survival of animals, plants and human beings (Muhammad Zafar et al., 2010). Mineral elements yield no energy, but they are necessary for smooth functioning of all the biological processes in an organism (Malhotra VK., 1998; Eruvbetine D., 2003). The importance of mineral elements in human, animal and plant nutrition is well recognized (Underwood EJ., 1971; Darby WJ., 1976). Plants form a major portion of the diet and play an important role in nutrition (Indrayan et al., 2005; Soetan et al., 2010; Kekuda et al., 2011; Dileep et al., 2013). The study of bioactive compounds of plant origin and assessment of elemental composition and concentration of the widely used medicinal plants is highly essential (Muhammad Zafar et al., 2010). According to the World Health Organization, determination of metals in medicinal plants is a part of quality control to establish their purity, safety and efficacy (WHO., 1992). *T. bellerica* is an important ethanomedicinal plant, belongs to the family “Combretaceae” (Elizabeth KM., 2005). It is a large deciduous tree up to 50m tall. Bark of the tree is ashy-grey covered with numerous fine longitudinal cracks and the inner bark is yellowish in colour (Deshmukh et al., 2019). In traditional Ayurvedic medicine, *T.bellerica* has been used as a "health-harmonizer" in combination with *Terminalia chebula* and *Emblica officinalis*. This combination is also used to lower down the cholesterol level and prevent from heart failure (Khotare Sneha and Rothe Sahadeo., 2016). Stem bark of *T. bellerica* possess potential compounds such as Arjungenin and its glycosides, belleric acid and bellericosides (Anand et al., 1997), hydrolysable tannins; gallic acid and ellagic acid in the water-soluble extract (Bade et al., 2010). Some sudies flew light on phytochemical and elemental composition of leaf and fruit but stem bark is untouched part in this regard. In the present study an attempt was made to determine phytoconstituents and the essential elements present in the stem bark of *T.bellerica.*

II. MATERIALS AND METHODS

Collection of plant material

T.bellerica (TB) stem bark was collected from the Botanical garden, Gulbarga University, Kalaburagi, respectively. These two plants were identified with the help of “Flora of Gulbarga district” HGUG-141 (Seetharam et al., 2000). The bark was thoroughly washed with double distilled water to eliminate contamination due to dust and environmental pollution, air dried and powdered using mixer grinder.

Preparation of crude extract

Preparation of crude extract was done by solvent extraction method. 10gm of plant power was soaked in saperate conical flasks containing 100ml of ethyl acetate, ethanol and methanol solvents, kept on shaker for 8-10 days and then it was filtered using whatman filter paper No.1. The filtrates obtained were used as test solutions for further preliminary screening tests.

Qualitative phytochemical screening tests

The preliminary phytochemical studies were performed for the evaluation of different chemical groups like alkaloids, flavonoids, phenols, terpinoids, glycosides, saponins, tannins and steroids, present in methanol, alcohol and ethyl acetate extracts of stem bark of *T.bellerica* by reffering standard procedures (Harborne JB., 1998).

Test for alkaloids

a. Dragendorff's test- To 2 ml of the extract 5 ml of distilled water was added; 2ml Hydrochloric acid was added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent extract was added. Formation of orange or orange red precipitate indicated the presence of alkaloids.

b. Mayer's test- To 2 ml of the extract taken in a test tube, a few drops of Mayer's reagent was added. Formation of a yellow / white precipitate confirmed the presence of alkaloids.

c. Wagner's test- 2 ml of the extract was acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown precipitate indicated the presence of alkaloids (Saldanha CJ., 1984).

Test for Phenols

a. Ellagic Acid test - The test solution was treated with a few drops of 5% (w/v) glacial acetic acid and 5% (w/v) $NaNO_2$ solution. The solution turned muddy or Niger brown precipitate occurred in the extract. It indicates the presence of phenol solution.

b. Ferric chloride test - 0.5 ml of $FeCl_3$ (w/v) solution was added in 2 ml of test solution, formation of an intense color indicates the presence of phenols (Memelink et al., 2001).

Test for flavonoids

a. Shinoda's test - In a test tube containing 0.5 ml of the extract 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicated the presence of flavonoids (Trease GE, Evans WC., 2002).

b. Ferric chloride test -Test solution with a few drops of ferric chloride solution shows intense green colour.

c. Zinc-Hydrochloric acid reduction test - Test solution with zinc dust and a few drops of hydrochloric acid shows magenta red colour(Trease GE, Evans WC., 2002).

d. Alkaline reagent test - Test solution when treated with sodium hydroxide solution, shows an increase in the intensity of yellow colour which becomes colourless on addition of a few drops of dilute acid (Trease GE, Evans WC., 2002).

e. Lead acetate solution test -Test solution with a few drops of lead acetate (10%) solution gives a yellow precipitate.

Test for triterpenoids

a. Liebermann - Burchard's test (LB test) - 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of a violet coloured ring indicated the presence of triterpenoids.

b. Salkowaski test - When a few drops of concentrated sulphuric acid were added to the test solution, shaken and allowed to stand, lower layer turns yellow indicating the presence of triterpenoids.

Test for saponins

a. Foam test - In a test tube containing about 5 ml of extract, a drop of sodium bicarbonate solution was added. The test tube was shaken vigorously and left for 3 minutes. Formation of honeycomb like froth indicated the presence of saponins.

Test for steroids

a. Liebermann-Burchard's test - 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of green colour indicated the presence of steroids.

b. Salkowaski reaction - 2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid was added slowly by the sides of the test tube. Formation of red colour indicated the presence of steroids.

Test for Tannins

a.Ferric chloride test - To 2ml of the extract, few drops of 5% w/v FeCl3 solution were added. A green colour indicated the presence of gallo tannins, while brown colour indicated the presence of pseudo tannins (Trease GE, Evans WC., 2002).

b.Gelatin test -1ml of test solution when treated with five drops of 1% gelatin containing 10% sodium chloride gives white precipitate. This confirmed the presence of tannins.

Test for glycosides

a. Baljet test -The test solution was treated with sodium picrate gives orange color

b. Kellar-Killiani test -1 ml of glacial acetic acid was carefully added to 2 ml of test solution of the extract and mixed well. Further, two drops of ferric chloride solution wad added after cooling. These contents were transferred carefully to a test tube containing 2 ml of concentrated H_2SO_4. A reddish brown ring was observed at the junction of two layers.

c. Raymond's test - Test solution when treated with dinitro- benzene in hot methanolic alkali, gives violet color.

d. Bromine water test -Test solution when treated with bromine water gives yellow precipitate.

e. Legal's test -Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red color.

Quantitative estimations of secondary metabolites

Estimation of Alkaloids

500mg powdered plant material was macerated with MeOH (Analytical grade) in mortar with pestle and centrifuged (2X). The supernatant collected was condensed to 1/4th volume and dilute acetic acid was added in a separating funnel. The acid layer was collected and 25ml of n-hexane and chloroform (1:1) mixture was

added and shaken well (3X). The chloroform layer is collected and washed with distilled water. Its p^H was adjusted to 11-12 by the addition of NH_4OH. The chloroform layer was separated and filtered using Whatman No. 1. The filtrate was finally transferred to a clean and pre-weighed beaker and dried under pressure at 40^0C for 6 h. The amount of alkaloid was calculated using the following formula (Ikan R.,1969).

$$\text{Total Alkaloids} = \frac{\text{Weight of Alkaloid residue (X)}}{\text{Weight of plant material (W)}} \times 100$$

Estimation of Flavonoids

500mg powdered plant material was homogenized with 10 ml methanol using mortar and pestle. Then, the homogenate was centrifuged at 3000 rpm for 20 min (2X). The supernatant collected was evaporated to dryness keeping in a hot water bath (80^0C). Thus, the residue obtained was redissolved in 5 ml distilled water. From this, 0.1 and 0.2 ml extracts were taken in test tubes and diluted to 2 ml with distilled water, 4 ml vanillin reagent was added to each tube rapidly. After 15 min the appeared brick red colour was read at 500 nm in the digital spectrophotometer against blank reagent. The standard curve was plotted using different concentrations of phloroglucinol as the standard flavonoids. The amount of flavonoids present in the sample was calculated with the help of the standard graph (Swain and Hillis1959).

Estimation of Tannin

500 mg of selected plant material was mixed with 75 ml distilled water in 250 ml conical flask and was heated gently and boiled for 30 min. Centrifuged at 2,000 rpm for 20 min and collected the supernatant in 100 ml volumetric flask and made up the volume. 1 ml of the sample extract was transferred to a 100 ml volumetric flask containing 75 ml of water. 5 ml of Folin Denis reagent was added and 10 ml of sodium carbonate solution and diluted to 100 ml with distilled water and shaken well. The blue colour intensity was measured in a spectrophotometer. The absorbance was read at 700 nm after 30 min and the 30 times dilution of the sample was made with distilled water and standard graph by using 0-100µg tannic acid. The tannin content of the sample was calculated as tannic acid equivalents from the standard graph expressed as mg/g (Schanderi SH., 1970).

Estimation of Phenols

500 mg of the selected sample was grinded with pestle and mortar in 10 times volume of 80% ethanol. The homogenated solution was centrifuged at 10,000 rpm for 20 min. The supernatant was saved and the residue was re-extracted with five times the volume of 80% ethanol centrifuged and pooled the supernatant. The supernatant was evaporated to dryness. Dissolved the residue in a 5 ml of distilled water. Pipette out 0.5 ml aliquots into test tubes, made the volume to 3 ml with distilled water. Added 0.5 ml Folin Ciocalteau reagent. After 3 min, added 2 ml of 20% Na_2CO_3 solution to each tube and mixed thoroughly. The test tubes were placed in boiling water for one min. cooled and the absorbance was read at 650 nm against reagent blank. A blue coloured complex was produced (molybdenum complex). A standard curve prepared using

different concentrations of catechol (Catechol was used as a standard phenol) (Sadasivam and Manickam., 1996).

Estimation of total Saponins

500 mg selected plant material was hydrolyzed by refluxing with 25 ml of 3N HCl at 60^0C for 4 h. The solid matter is retained on the Whatman No. 1 filter paper and further washed it with half diluted aqueous NH_4OH until the washings were neutral (P^H 6.8-7.0). Then, the residue was dried and extracted for saponins in the Soxhlet extractor using chloroform for 6 h from this; 1 ml extract was taken and evaporated to dryness in vaccum. Thus, the residue obtained was re-dissolved in 4 ml H_2SO_4 and methanol reagent. The absorbance was read at 405 nm in UV/VIS spectrophotometer against a blank, after allowing the reaction to proceed for 2 min, which is optimum time required for the chromophore to develop a stable optical density. The amount of saponins present in the plant material was calculated (Sanchez et al., 1972).

Estimation of Cardiac Glycoside

25g of plant powder in 200ml of 70% ethanol is taken then it is kept in shaker at 300rpm for 6hrs at room temperature, then filter it and add 500ml of distilled water followed by 100ml of 12.5% lead acetate (to precipitate tannins,resins and pigments). Volumes were made to 800ml with distilled water and keep it on shaker at 300rpm for 10mins, add 200ml of 4.77% disodium hydrogen phosphate(Na_2HPo_4) solution to precipitate excess pb++ ions. Then the above solution were filtered and evaporated to dryness. Then calculate the % of glycoside using the formula.

$$\%\text{of Glycoside} = \frac{\text{weight of dried extract}}{\text{weight of plant material}} \times 100$$

Estimation of Steroids

1ml of plant extract is added to 4ml of chloroform and then add 2ml of Libberman-Burchard reagent and mix it well. Then the test tube were covered with black paper and kept it under dark for 15mins. The green color complex formed was measured using spectrophotometer at 640nm.

Estimation of Terpinoids

Terpenoids content was determined by the procedure of Ferguson, 1956. About 500 mg bark powdered was taken and soaked in alcohol for 24 hours. It was filtered and filtrate extracted with petroleum ether; this ether extract was treated as total terpenoids (Kim et al., 2003).

III. RESULTS AND DISCUSSION

In the present investigation phytochemical analysis of methanol and ethanolic crude extracts of stem bark of *T.bellerica* revealed the presence of Alkaloids, flavanoids, phenols, terpinoids, saponins, steroids, tannins and glycosides. Where as the ethyl acetate extract showed the presence of all the phytochemicals except saponins, steroids and tannins which is represented in Table 1.

Table 1: Priliminary phytochemical screening of different solvent extracts of stem bark of *T. bellerica.*

ytochemical Tests	Methanol	Ethanol	Ethyl acetate
Alkaloids			
Dragendorff's test	+	-	+
Wagner's test	-	+	+
Mayer's test	+	+	-
Flavonoids			
Shinoda test	+	+	+
Ferric chloride test	+	-	-
Zinc-Hydrochloric acid reduction test	-	+	+
Alkaline-reagent test	+	+	-
Lead acetate test	+	+	+
Phenols			
Feric chloride test	+	-	+
Ellagic acid test	+	+	-
Terpinoids			
Liebermann-Bur chard's(L-B) test	-	+	-
Salkowaski test	-	+	+
Saponins			
Foam test	+	+	-
Steroids			
L- B test	+	+	-
Salkowaski test	+	-	-
Tannins			
Ferric chloride test	+	-	-
Gelatin test	+	+	-
Glycosides			
Baljet test	+	+	-
Keller-Killiani test	-	+	+
Raymond's test	-	-	+
Bromine water test	+	+	-
Legal's test	+	-	+

+ve - Presence of compound; -ve - Absence of compound.

Similarly, quantitative estimation study reveals the presence of high quantity of glycosides (1.86mg/g), flavanoids (1.28mg/g), phenols (1.24mg/g), alkaloids (1.20mg/g) and terpenoids (1.12mg/g) and the least quantity of steroids(0.84mg/g), saponins (0.58mg/g) and tannins (0.38mg/g) were observed and is represented in Table 2.

Table 2: Quantative estimations of secondary metabolites from the stem bark of stem bark of *T.bellerica*

Secondary metabolites	Concentration mg/g
Alkaloids	1.20
Flavanoids	1.28
Tannins	0.38
Phenols	1.24
Saponins	0.58
Glycosides	1.86
Steroids	0.84
Terpenoids	1.12

Similar studies were also reported on water extracts of *T. bellerica* fruit indicated the presence of flavonoids, hydrolysable tannins, saponin and terpenes (Khosit Pinmai et al., 2010). In another study petrolium ether, chloroform, ethanol and water extracts of fruit showed the presence of triterpenoids, saponins, carbohydrates, glycosides and tannins (Sarabjit kaur and Jaggi., 2010). According to Arif-Ullah Khan and Anwarul Hassan Gilani (2008)- study revealed the presence of flavonoids, sterols and tannins in the crude extract of fruit. Where as, Iqbal Ahmad and Arina Beg (2001)- also reported phenols, tannins, flavonoids as major active constituents. Similarly, phenolics, alkaloids, flavonoids and tannins were also reported from fruit (Nithya devi et al., 2014).

According to some study, fruits various parts such as seeds was evaluated for very poor quantity of phenolics (0.65 Gallic Acid Equivalent (GAE)) and flavonoids (0.77 Quercetin Equivalent (QE)) are present compared to epicarp (139.05 GAE and 141.26 QE) and mesocarp (135.23 GAE and 142.05 QE) (Kalyan hazrat., 2019). The seeds of *T. bellerica* possess alkaloid, phenols, steroids and tanins in the methanol and ethylacetate extract. The Aqeuous and chloroform extract showed the presence of flavoids, saponon and tannins (Arul Amutha Elizabeth et al., 2017). Fruit revealed the presence of Alkaloid, antraquinone glycoside, saponins, flavonoids, polysaccharides, steroid, tannin, phenol, carbohydrates and proteins (Meena et al., 2010; Asthana et al., 2011; Devi et al., 2014) . Leaf showed the presence of Proteins, steroids and terpinoids, saponins, tannins, amino acids, alkaloids, carbohydrates and flavonoids(Prabhu et al., 2012; Shankar et al., 2014).

A total of fourteen elements were recovered from the stem bark of TB. The concentration of Calcium is 14.1266mg/L, Pottassium is 7.3965mg/L, Magnesium 6.4859mg/L, Iron is 0.4571mg/L, Copper is -0.0023mg/L, Zinc is 0.1061mg/L, Manganese is 0.1492mg/L, Chromium is -0.0930, Aluminium is 0.3078mg/L, Cadmium is 0.00123mg/L, Silicon is 30.0578mg/L, Molybdenum is 0.4118mg/L, Titanium is 1.2392mg/L and Vanadium is 0.1638mg/L were recorded and the results are represented inTable 3.

Table 3: concentration of mineral content in stem bark of *T.bellerica*

Sl.No	Elements	Concentration (mg/L)
1	Copper	-0.0023
2	Iron	0.4571
3	Zinc	0.1061
4	Pottassium	7.3965
5	Magnesium	6.4859
6	Manganese	0.1492
7	Cadmium	0.0123
8	Silicon	30.0578
9	Vanadium	0.1638
10	Titanium	1.2392
11	Calcium	14.1266
12	Chromium	-0.093
13	Molybdenum	0.4118
14	Aluminium	0.3078

In the present study, as compared to earlier reports, high concentration of calcium (14.1266 mg/L in TBS) and magnisium (6.4859mg/L in TBS) content were observed. However, the calcium concentration reported in the fruit of *T.bellerica* was 0.12mg/kg and magnisium content was 0.38mg/kg (Morabad et al., 2013). Similarly, the elemental analysis of four Terminalia species were carried out using three different parts such as leaves (TBL), seed kernel (TBSK) and seed coat (TBSC). The *T.bellerica* showed the presence of all the elements except alluminium in seed keral and seed coat of this plants. The element Titanium is completely absent in all the three parts of TB (Suryakant Chakradhari et al., 2019) but in this study 1.2392mg/L of Titanium was recorded. In present study maximum amount of silicon (30.0578mg/L) was recorded. Silicon is present in all body tissues, but the highest concentrations of silicon is present in bone and other connective tissue including skin, hair,arteries, and nails (Jugdaohsingh R., 2007). Diets containing more than 40mg/day of silicon have been positively associated with increased femoral bone mineral density compared to dietary intake of less than 14mg/day (Jugdaohsingh et al., 2004). Very few reports available on phytochemical analysis of this plant and as for my knowledge there is no report on mineral analysis of *T.bellerica* stem bark. so this might be the first report on this plant. Further study is necessary to explore the phyto-pharmacological efficacy of stem bark of *T.bellerica.*

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