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Qualitative And Quantitative Phytochemical Screening of Secondary Metabolites in *Terminalia Arjuna* (Roxb.) Wight & Arn

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Abstract:

Present investigation deals with the qualitative screening and quantitative screening of secondary phytocompounds in bark of important medicinal crude drug Terminalia arjuna (Roxb.) Wight& Arn Qualitative phytochemical screening was carried out to identify the different classes of secondary metabolites in various chemical extracts such as methanol, ethanol, Petroleum ether, chloroform, acetone and water. Phytochemical analysis of the extracts proved the presence of secondary phytocompounds such as Alkaloids, Flavanoids, Saponins, lipid, Terpenoids, Tannins, steroids and starch. Quantitative phytochemical screening showed that total alkaloids and total tannin was more 18.2±3.65 mg/g and 16.07±0.02 mg/g in bark however total flavonoid was estimated in bark is 5.523±0.007mg/g and total saponin content is 4.612±0.010mg/g.

Keywords: Terminalia arjuna, Secondary Phytocompounds. Qualitative and Quantitative screening

Introduction:

The Bhimashankar Wildlife Sanctuary (BWS) is one of the wildlife sanctuaries located along the northern Western Ghats in Maharashtra. It was notified on 10 October 1985 by the Government of Maharashtra (1985) as a step to conserve the state animal, the Giant Squirrel *Ratufa indica* spp. elphinstonii. The sanctuary is named after the Bhimashankar temple (one of the twelve jyotir-linga (self-emerged) Shiva temples in the country) located inside the sanctuary and surrounded by a sacred grove. It is rich in Flora (Herb, Shrubs, Climbers, trees, Medicinal Plants) as well as Fauna. Jagdale (1994) studied the ecology of the BWS area and concluded the ecosystem of the sanctuary is unique and very fragile, so it requires priority for conservation and minimize local use of natural resources and documentation of some ethno-medicinal plants of BWS area. Rahangdal *et al*., (2017) focus on Florristic diversity of Bhimashankar Wildlife Sanctuary.

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids [5,11]. These bioactive compounds are synthesized by primary and secondary metabolism of living organisms. Secondary metabolites are chemotaxonomically diverse compounds with obscure function. They are widely used in the human therapy, veterinary, agriculture, scientific research and countless other areas [17]. A large number of phytochemicalsbelonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*

[3]. Bioactive compounds can be derived from barks, leaves, flowers, roots, fruits, seeds [8]. [9,10,11]. In the present work, qualitative and phytochemical analysis were carried out in five plants of Bhimashankar Wildlife Sanctuary, Pune.

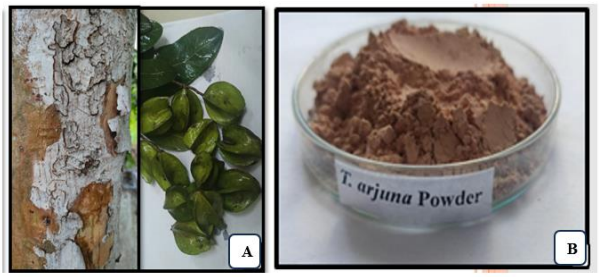


Figure 3.1: A) *T. arjuna Bark & fruit*
B) *T. arjuna bark* powder

Materials and Method:

Collection and Processing:

Bark of *Terminalia arjuna* was collected from tribes of Bhimashankar Wildlife Sanctuary, Pune, (MS) India during the season in the month of March- April 2023. The plant was identified with help of botanist, identified & authenticate from Agharkar Research Institute, Pune. This bark sample was washed thoroughly with tap water, shade dried and grinded finely into the powder form, which was then used for various form of extract preparation.

Preparation of Extract:

10g per 100ml of each solvent such as methanol, ethanol, Petroleum ether, chloroform, acetone and water extracts of bark of *Terminalia arjuna* were prepared separately with the help of cold ectraction method. After extraction the extracts were filtered through Whatman No. 41 filter paper. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard preliminary phytochemical methods given by (Trease & Evans, 2009; Harbone, 1998; Kokate *et al.,* N. Raaman ,2006)

Qualitative Phytochemical Screening of secondary phytocompounds:

Test for Alkaloids: Mayer's test: Extract was treated with few drops of Mayer's reagent. The white or pale precipitate indicated the presence of Alkaloids.

Test for Terpenoids: Salkowski test: 2 ml of each extract was mixed with 2ml of chloroform and concentrated H2SO4 (3ml) was carefully added to form a layer. An appearance of red colour indicated the presence of steroids

Test for Tannins: Ferric chloride test: The ethanolic extract is treated with 2 ml of FeCl3 solution. The blue-black precipitation is observed.

Test for Saponins: Foam test: 5ml of filtrate was diluted with 20ml of water and vigorously shaken. The test tube was observed for the presence of stable foam upon standing.

Test for Flavonoids: Sodium hydroxide test: Few quantities of each portion was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid which indicated the presence of flavonoids.

Test for Starch: Take 1 ml of plant extract. add drops of iodine solution for some time. If it turns blue-black afterwards, it contains starch.

Test for Lipid: Take a small amount of the sample in a test tube. Add a few crystals of potassium bisulfate (KHSO4). Heat the mixture gently and then strongly. A pungent, irritating odour of acrolein indicates the presence of glycerol or fats.

Test for Steroids: Dissolve 1 ml plant extract in 2 ml chloroform. Carefully add few drops of concentrated sulfuric acid along the sides of the test tube, allowing it to form a layer below the chloroform layer. A reddish-brown colour at the interface between the two layers indicates the presence of steroids.

Quantitative Phytochemical Screening:

Determination of total alkaloid contents: The total alkaloids contents were determined using Bromocresol Green Method. The presence of alkaloid was qualitatively tested using Mayers reagent. The alkaloid presence was confirmed by the yellowish white precipitate produced when Mayers reagent was applied. Quantitative estimation of alkaloid was done using UV-VIS spectrophotometer. This approach is dependent on the interactions amid alkaloid and bromocresol green (BCG) dye. Soxhlet extract of 925 mg was dissolved in 2 N HCl and then filtered. 1 mL of filtrate was added into separatory funnel then rinsed with chloroform in three separate intervals. The pH was adjusted to neutral using 0.1 N NaOH. Then, 5 mL of bromocresol green (BCG) and 5 mL of phosphate buffer was combined, the mixture was agitated and further extracted with chloroform through vigorous shaking. The mixture was then transferred into a volumetric flask and diluted with chloroform. The absorbance was measured at 470 nm. Atropine was used as a standard to quantify total alkaloids contents.

Determination of total flavonoid content (TFC): The total flavonoid contents (TFC) of bark of *Terminalia arjuna* was assessed through a colorimetric method involving aluminium chloride (Singh *et al.*, 2019). In this process, 300 µL of the sample was combined with 150 µL of a 0.3 M AlCl3, 150 µL of 0.5 M NaNO2, and 3 mL of 30% aqueous methanol in a test tube. Subsequently, 1 mL of 1 M sodium hydroxide (NaOH) was added. The absorbance was then measured at 506 nm using a UV- VIS spectrophotometer The TFC was calculated and expressed as milligrams of rutin equivalent per gram of dried extract (mg RE/g).

Determination of total Tannin Content: The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mi of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Determination of Total Saponin Content: Estimation of Total Tannin Content [15, 16] The tannins were determined by Folin-Ciocalteu method. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu phenol reagent, 1 mi of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible

spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Results And Conclusion:

Sr. No.	Solvent system	% Yield of extracts
1	Methanol	19%
2	Ethanol	10%
3	Petroleum ether	0.6%
4	Chloroform	2.12%
5	Acetone	4.75%
6	Water	15%

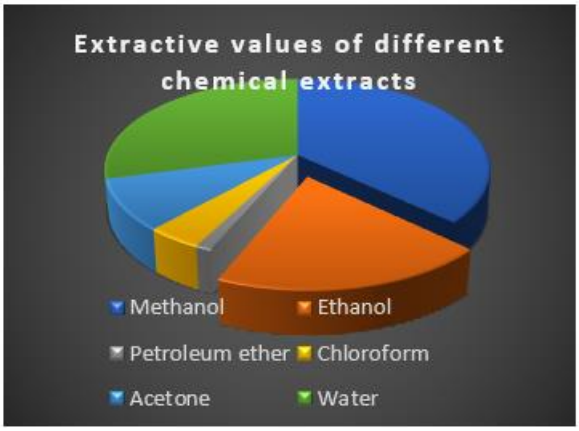
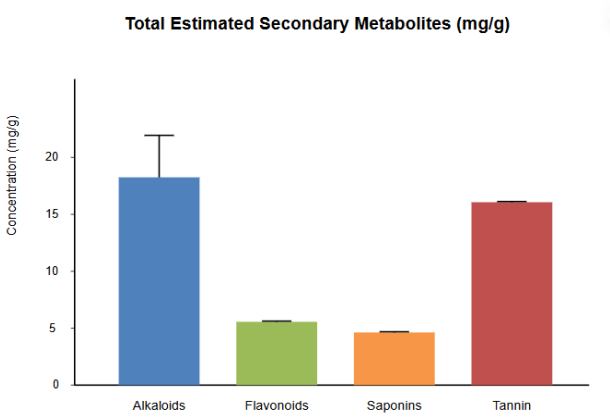


Table & graph 1: Extractive values of different chemical extracts of bark of *Terminalia arjuna*

Table 2: Preliminary phytochemical screening of bark extract ss *arjuna* (Roxb.) Wight& Arn (+ Present - Absent)

Sr. No.	Presence/absence of bioactive components	Test	Methanol	Ethanol	Petroleum ether	Chloroform	Acetone	Aqueous
1	Alkaloids	Mayer's test	+	+	-	-	+	-
2	Saponins	Foam test	-	-	-	-	-	+
3	Tannins	Ferric Chloride test	+	+	-	-	+	+
4	Steroids	Salkowski test	+	+	-	-	+	+
5	Flavonoids	Alkaline Reagent test	+	+	-	-	+	-
6	Terpenoids	Salkowski test	+	+	-	-	+	+
7	Lipid	Acrolein test	+	+	-	-	+	+
8	Starch	Iodine test	+	+	-	-	+	-



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