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Preliminary phytochemical analysis and antimicrobial potential of *Terminalia arjuna (Roxb)Wight & Arn.*

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Abstract
The aim of our current study was to explore the Preliminary photochemical analysis and antimicrobial potential of the diverse extracts of bark of the *Terminalia arjuna* plant. The diverse extracts such Acetone, Ethanol, aqueous extracts and petroleum ether exhibits comparable antimicrobial activity with the control. Some universal and available standard tests were done in order to test phytochemical screening. Agar well diffusion method was used to carry out antimicrobial bioassay. Five crude extracts were organized from the bark of the plant *Terminalia arjuna* by soxhlet method using different solvents. The extracts were subjected to screening in order to detect preliminary photochemical analysis and possible antimicrobial activity against *E. coli, Salmonella typhi, Klebsiella pneumonia, Staphylococcus aureus* and fungal strain *Candida albicans* as standard using the agar well diffusion method. Phytochemical screening showed the active compounds presence in elevated concentration. The active compounds included flavonoids, phytosterol, phenolic-compounds, tannins & glycosides and lactones. The extract's antimicrobial activity showed superior inhibition zone against gram-negative bacteria. The *Terminalia arjuna* bark extract exposed the presence of bio-active constituents. These constituents are known to show signs of physiological and medicinal activities.

Keywords: phytochemical analysis, *Terminalia arjuna*, antioxidant, antimicrobial activity, total flavonoid

Introduction
Terminalia arjuna (*T. arjuna*), has height of about 25 to 30 mtr tall and diameter (around breast height) of 2 to 2.5 mtr, often having a buttressed trunk, is basically a large-sized deciduous tree. *T. arjuna*. This plant belonging to the family Combrataceae. Habitually this particular tree belongs to the evergreen habit, but strangely it is consider as an exotic to India. This tree is habitually an evergreen tree and is considered exotic in India. It is found all through the South-Asian region. Old or mature leaves shades of in the middle of summer and new leaves appear in the middle to end of summer.

Classification
Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Myratales
Family: Combrataceae
Genus: *Terminalia*
Species: *arjuna*
Vernacular Name: Arjunsadada

We can say that, *Terminalia arjuna* is celebrity tree in the Ayurveda medicine. It shows wide spectrum biological activity. Mostly bark of the tree is consider as antipyretic, lithotriptic, hypolipidemi, anti-dysentric, astringent, cardio-tonic, anticoagulant and antimicrobial (Mandal *et al*., 2010) [6]. It is also used as antiuremic agent (Das *et al*., 2011)[4]. Many useful phyto-constituents have been secluded from *T. arjuna*, which included flavonoids and tannins for its anticancer, triterpenoids for cardiovascular properties, antimicrobial properties and so on (Nema *et al*., 2012). In cirrhosis of liver the powder of bark gives relief in symptomatic hypertension by acting as a diuretic (Chatterjee A.S. 1994)[3]. In studies involving mice, its leaves have been shown to have anti-inflammatory and analgesic properties (Biswas *et al*., 2011) [1]. We have undertaken this study to ascertain the preliminary phyto-chemical screening of the extract and determination of antimicrobial and antioxidant activity. Mainly from the bark of tree.
According to World Health Organization, the best source to obtain variety of drugs would be medicinal plants (Yadav and Agrawala 2011). [16] This plant is highly medicinal and widely used by Ayurveda physicians because of its curative properties in heart problems including deposits in arteries, hypertension and angina. Mainly bark is consider as a medicinal potential and used in fever, contusion and astringent. Bark is styptic, febrifuge, tonic

and anti-dysenteric; pulverized bark act as a diuretic in in the liver (Mandal *et al*., 2010) [6]. Juice of leaves is used as a remedy in curing ear ache. *Terminalia* may be protective against gastric ulcers, in addition to its cardiac effects. Oligomeric proanthocyanidins and flavonoids present in the *Terminalia* considered as a positive inotropic effects.

In living organisms primary or secondary metabolism produces the bioactive phytochemicals which are necessary in the medicine. Secondary metabolites are taxonomically and chemically tremendously diverse compounds with difficult to understand functions. They are widely used in the scientific research, human therapy, agriculture, veterinary and countless other areas (Vasu *et al*, 2009) [14]. Medicinal plants play a significant role in the prevention of various degenerative diseases. This is because they have active chemical constituents with high antioxidant property (Lukmanul *et al*., 2008) [5] and they therefore even offer many useful benefits to mankind. In *in-vitro* conditions large no of chemical compounds relating to the several important phytochemical have shown inhibitory effect on the microorganisms. Botanical medicines refer to the material i.e. any part of the plant ex. Leaves, bark, stem, fruits, flowers, roots for medicinal purposes. Having information of the chemical constituents of plants is advantageous as such information will be valuable for the synthesis of complex chemical substances (Mojab *et al*., 2003) [7]. In ancient India, various critical diseases were prevented with the use of medicinal plants. Plant kingdom is source of many vital drugs. Botanical plants possesses many organic compounds which are vital for the human body. In modern age there is increasing attentiveness towerds the use of herbal medicines. As it has no side effects. According to Ayurveda, in traditional Ayurvedic medicine, *Terminalia arjuna* has been quoted as a source which can be used to balance the three "humors": pitta, vata and kapha. The bark of *Terminalia arjuna* has widely been used in India for more than 3000 years, chiefly as a heart remedy. It has also been used for bile duct disorders, asthma, poisonings and scorpion stings. It is also used as a nervine tonic. *Terminalia arjuna* (Roxb.) Wight & Arn. showed good anti diabetic activity (Kharkar *et al*., 2013) [12]. Therefore by considering immense medicinal importance of the plant the present paper reports work on preliminary phytochemical screening and antimicrobial investigated on *Terminalia arjuna* (Roxb.) Wight & Arn.

Materials and Methods

Collection, identification and processing of plant material

The bark of *T. arjuna* was cut and taken from Anjaneri village nearby area of Nashik. Plant was correctly identified with the help of Maharashtra's flora and also with the flora of Nashik District. Collected plant material was washed under tap water and then dried in sun-shade. It was then grinded down to fine powder texture with electric blender and preserved in airtight bottles. This sample was used for extraction of organic compound.

Extraction of organic crude material from *Terminalia arjuna* (Roxb.) Wight & Arn.

50 gm of plant bark powder sample weighted and used for soxlation.

Solvent Used

Depending on polarity the following solvent selected

1. Distilled water
2. Ethyl alcohol
3. Hexane
4. Petroleum ether
5. Methanol.

Phytochemical analysis of plant extract: Metabolism and chemical processes of plant bodies are majorly driven by phytochemicals. These phytochemical then studied included flavonoids, alkaloids, glycosides, terpenoids & steroids, tannins & saponin.

Phytochemical primary Identification test: Detection test were done to find out active secondary metabolites such as glycosides, alkaloids, terpenoids, steroid, flovonoids, tannin and saponim by the following procedures.

Alkaloids

Detection of alkaloids (Evans, 2002): Solvent free 50 mg extract is stirred with few ml of dilute hydrochloric acid. After that his mixture is then filtered and tested cautiously with different alkaloid reagent as per below test.

a. Mayer's test: A drop or few drops of Mayer's reagent are added to a few ml of filtrate alongside the test tube. Positive result indicates that a creamy or white precipitation observed.

Mayer's Reagent: 1.358gm of mercuric chloride dissolved in 60ml of water. Stirred simultaneously. While stirring we have add 5.0gm of potassium iodide in 10ml D.W. Add these two solutions in 100ml.

b. Wagner's Test (Wagner, 2004): Wagners reagent few drops are added to a few ml of filtrate alongside of the test tube. Presence of a reddish brown precipitate indicates a positive test.

Detection of Carbohydrates and Glycosides: - 100gm plant extract is in 5ml of water and filtered.

a. Mayer's test: Add 2ml of plant filtrate in 2 drops of alcoholic solution add 1-napthol, mix it and add 1 ml concentrated sulphuric acid slowly. End point violent ring indicates the presence of carbohydrates.

b. Barrfoed's test: 1 ml of Barfoed's reagent is added to 1 ml of filtered solution and is steadily heated for 2 min on a boiling water bath. Occurrence of a red precipitate indicates the presence of sugar in the filtered solution.
Barfoed's reagent: 30.5gm of copper acetate is dissolved in 1.8ml of glacial acetic acid.
c. Benedict's test: Benedict's reagent 0.5 ml is added to 0.5 ml of filtered solution and mixture is steadly heated for 2 min on a boiling water bath. End result observed colored precipitate shows the presence of sugars in the filtered solution.
d. Iodine solution test: Iodine solution 1 ml added to 3 ml of aqueous extract. Occurrence of purple colour is end point. It indicates the presence of carbohydrates.
e. Keller Kiliani's test: 2 ml of extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was then poured into the test tube containing 1 ml of concentrated sulphuric acid. A brown ring at the interphase indicates the presence of deoxe sugar, characteristics of cardenolides.

Detection of Saponin
50 mg plant extract was diluted in D.W.. Water is added till 20ml of solution is formed. For 15 min, this solution is shaken in a graduated cylinder. Occurrence of 2 cm layer of foam ascertains the presence of saponin.

Detection of amino acids and proteins
Distilled water 10 ml is used to dissolve 100 mg of extract and the resultant solution is then filtered through whatman filter paper No. 1. Then the filtrate is subjected to test for amino acids and proteins.
a. Million's test: Million's reagent few drops are added to 2 ml of filtrate. Observed white precipitate ascertains of proteins.
b. Ninhydrin test: 2 drops of ninhydrine solution in 2 ml of aqueous filtrate. The final result occurrence of a charachteristic purple color shows the presence of amino acids.

Detection of Tannins and phenolic compounds
a. Ferric chloride test: Plant extract 50 m dissolve in 5 ml of distilled water. Added 5% of neutral ferric chloride solution drops in that aqueous extraxt. The end point shows dark green color ascertains the presence of phenolic compounds.

Detection of Gum and Mucilage: In 10 ml of distilled water 100 mg of plant extract dissolve. We have to stir until white ppt and mucilage appears after adding the 25 ml alcohol.

Glycosides: The compounds glycosides which when subjected to hydrolysis, give rise to one more sugar (glycones) and a compound which isn't a sugar (aglycone or genine). To the solution of this extract in glacial acetic acid we added a few drops of concentrated sulphuric acid and ferric chloride. We then observed for, in upper layer bluish green colour and at junction of two layers reddish colour appears.

Terpenoid and steroids: 4 mg of extract was mixed with 0.5 ml of acetic anhydride and 0.5 ml of chloroform. Then concentrated sulphuric acid solution was added slowely and red violet color developed for presence of steroid.

Flavonoids: 4 ml of extract solution was treated with 50% of 1.5 ml of methanol solution. The extract solution was warmed and added pinch of metal magnesium. After that in solution 5 – 6 drops of concentrated hydrochloric acid was added and subsequent red color shows presence of flavones.

Tannins: Few drops of ferric chloride solution and 1 ml of water to 0.5 ml of plant extract solution. If Greenish colour appear indicates catecholic tannins and if Blue colour appears shows Gallic tannins.

Fats and fixed oils: A small quantity of plant extract was taken and squeezed between two filter papers. If occurrence of oil stains on the paper ascertains presence of fixed oil in plant extract.

Saponification test: 0.5 N alcoholic potassium hydroxide solution few drops added to miniscule quantity of extract in conjunction with drops of phenolphthalein indicator. The mixture is gradually heated on water bath for over 2 hours. Formation of soap partial neutralization of alkali ascertains the presence of fats and fixed oils.

Determination of total flavonoids content
Flavonoids determination carried out by aluminum chloride colorimetric method as shown by Wang S.Y., Jiao H. 2000[15]

D) Antimicrobial Activity
Inoculum: The microorganism isolated and incubated at 35+2 ^{0}C for 4 hrs. The turbidity of the resulting bacterial adjusted to turbidity equivalent to 1 McFarland turbidity standard. This level of turbidity is equivalent to approximately 3.0 X 108 CFU/ml.

Bacterial strain used

To study antimicrobial activity on the following one fungal strain and four bacterial strains:

- *Escherichia coli (ATCC25922)*
- *Klebsiella pneumonia (ATCC 25922)*
- *Salmonella paratyphi*
- *Staphylococcus aureus (ATCC25923)*
- *Candida albicans*

Agar well diffusion method

The agar well diffusion method was employed Muller – Hinton agar plate were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60^0 each time to ensure even distribution of inoculums. As a final step the rim of the agar was also swabbed. After ensuring that the inoculums have dried at room temperature, 6 mm diameter wells were bored in the agar. Each extract was checked for antimicrobial activity by introducing 100 µl of 4000 µg/ml concentration simultaneously into triplicate well and respective solvent were used as the dilution medium for positive control. The plates were made to rest at room temperature for an hour. This allowed through extract to diffuse well into agar. They were incubated at 35 $\pm$ 2° C for 24 hours. Solvent extracts were shown area of inhibition concentration by using 25µl, 50µl, 75 µl, 100µl and 125 µl against positive control using pure solvent of 100 µl. The plates were allowed to rest at room temperature for about an hour for the extract to diffuse in to the agar and they were then incubated at 35 $\pm$ 2° C for 24 hr. The zone of inhibition was measured with scales and subsequent observations were noted in notebook.

Result and Discussion

To maintain the health of individuals of various communities medicinal plants are playing a key role. (Pascaline *et al*., 2011) [11]. The various researcher carried out the phytochemical analysis of the plant extract for detecting the medicinally physiochemical important phyto-constituents (R. John Wiley and Sons, 1993) [13]. Analysis of plant extract shows proteins, carbohydrates, phenols, tannins, flavonoids, saponins, glycosides, steroids, terprnoids and alkaloids. Plant bark powder subjected to successive extraction with various solvent like Hexane, Methanol, ethanol, petroleum ether and distilled water. Present study on phytochemical investigation results has been shown in Table 1. The different solvent based crude extract revealed presence of phytochemical like carbohydrates, saponin, alkaloids, steroids, tannins and phenols etc. in table no 2 and 3. All solvent based extract showed detection of presence of alkaloid. Metanol based extract strongly showed presence of carbohydrates while aqueous extract showed presence of saponins. Etanolic extract resulted presence of phenolic compounds. Fixed and volatile oil strongly presence in all extract except in aqueous extract. The investigated phytochemical were bioactive and its show various pharmacological activities. They specifically used to cure variety of disease like hemicranias, jaundice, hepatopathy, diabetes, leprosy, fever, pectoralgia, cough, gastropathy, hernia, hemorrhoids, helminthiasis, dyspepsia and skin diseases. It is also used as a nervine tonic. *Terminalia arjuna* (Roxb.) Wight & Arn. bark powder known for good anti diabetic agent (R. Kharkar *et al.*, 2013) [12]. *Terminalia arjuna* (Roxb.) Wight & Arn plant four extract aqueous extract, acetone, ethanol and petroleum ether tested against human pathogenic bacteria and fungi. Out of this four extracts, ethanol, acetone, petroleum ether, showed high *in vitro* antibacterial activity against *S. aureus*, acetone extract showed strong antibacterial activity against *K. pneumonia* and C. *albicans* fungal pathogen strongly inhibited. Ethanol extract of bark resulted inhibitory activity against *E.coli* and S. *aureus* human pathogenic bacteria. *S. paratyphi* growth suppressed by petroleum ether, further noted that pure solvent used as control not showed any activity against micro organism.

Table 1: Physicochemical Characteristics of Different Extracts of *Terminalia arjuna* (Roxb.) Wight & Arn.

Sr. No.	Solvent used	Initial weight of powder in grams	Final weight of powder in grams	Weight of crude extract in grams	Color of Extract
01	Distilled water	50	48.55	1.65	Dark brown
02	Ethanol	50	47.85	1.47	Dark Green
03	Acetone	50	47.95	1.43	Green
04	Pet. Ether	50	46.95	1.23	Pale green

Table 2: Preliminary phytochemical analysis of T. arjuna's bark extract.

Sr.No.	Phytoconstituents	Tests	Conclusion
1	Alkaloids	Dragendroff's test	++
2	Carbohydrates	Molisch's test	+
3	Flavanoids	Lead Acetate test	+
4	Glycosides	Keller-Killiani test	++
5	Lactones	Legal's test	++
6	Tannins and Phenolic Compounds	5% $Fecl_3$ Test	++

7	Phytosterols	Salkowski reaction	++
8	Proteins	Ninhydrin test	+
9	Saponins	Foam test	+
10	Triterpenoids	Liebermann-Burchard's test	++

Table 3: Preliminary phytochemical analysis of crude extract of *Terminalia arjuna* (Roxb.) Wight & Arn.

Sr.No	Phytochemical Test	D.W.	Ethanol	Acetone	Pet. Ether
1.	a. Mayers Reagent	+	++	++	++
	b. Wagners Reagent reagent	++	++	++	++
2.	a. Molish Test	+	++	+	++
	b. Barfoed's test	++	++	++	+
	c. .Benedict's test	-	+	+++	-
3.	a. Foam test	+	+	-	+
4.	a. Million's test	+	++	++	+
5.	a. Ferric test	++	++	-	-
6.	a. 95% alcohol	-	+++	+	+
7.	a. Spot test	-	-	-	
8.	Flavonoid	+	+	++	+
9.	Glycosides	++	+	+	+

+ Low concentration
++ High concentration

Table 4: Antimicrobial Activity of *Terminalia arjuna* (Roxb.) Wight & Arn.

Sr.no	Microorganis strain		Extract of *Terminalia arjuna*					
		Zone of inhibition in cm						Control
1.	*E. coli*	Conc. Of Extract in µl	25	50	75	100	125	
		Distilled water	Nil	Nil	Nil	0.2	0.3	Nil
		Ethanol	Nil	0.1	0.2	0.4	0.5	Nil
		Aceton	Nil	Nil	Nil	0.3	0.4	Nil
		Pet. Ether	Nil	Nil	Nil	0.2	0.4	Nil
2.	*S.aureus*							
		Distilled water	Nil	0.1	0.2	0.3	0.5	Nil
		Ethanol	Nil	0.2	0.3	0.3	0.4	Nil
		Aceton	0.1	0.2	0.4	0.6	0.7	Nil
		Pet. Ether	Nil	Nil	0.2	0.3	0.5	Nil
3.	*K. pneumonia*							
		Distilled water	Nil	0.1	0.3	0.3	0.4	Nil
		Ethanol	Nil	Nil	0.1	0.2	0.3	Nil
		Aceton	Nil	0.1	0.3	0.4	0.6	Nil
		Pet. Ether	Nil	01	0.2	0.3	0.4	Nil
4.	*S.paratyphi*							
		Distilled water	Nil	Nil	Nil	0.2	0.3	Nil
		Ethanol	Nil	Nil	0.3	0.4	0.6	Nil
		Aceton	Nil	Nil	0.1	0.2	0.3	Nil
		Pet. Ether	0.1	0.2	0.3	0.4	0.5	Nil
5.	*C. albicans*							
		Distilled water	Nil	Nil	Nil	0.1	0.3	Nil
		Ethanol	Nil	Nil	0.1	0.2	0.3	Nil
		Aceton	0.1	0.2	0.3	0.4	0.5	Nil
		Pet. Ether	Nil	0.1	0.2	0.3	0.6	Nil

Conclusion

Current study concluded in the finding bark of *Terminalia arjuna* consis of different types of useful chemical compounds such as phyto-serols, flavonoids, phenols, tannis, alkaloids and saponins. Flavonoid antioxidant activity is important. Mainly Flavonoid compounds of *Terminalia arjuna* are responsible for the anti-microbial and anti-oxidant activities. Hence we can say that the bark of this particular tree is a capable source of therapeutic agents against variety of pathogenic infections. The traditional medicine practice is recommended strongly for these plant as well further studies carried out isolation and purification of medicinally important compounds is useful for various pharmacological activities and it's also help curing the various diseases.

References

1. Biswas M, Biswas K, Karan TK, Bhattacharya S, Ghosh AK, Haldar P K. Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. *J Phytol*,2011:2011:3(1):33–38.
2. Brown JE, Rice-Evans CA. Luteolin rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. *Free Radic Res*,1998:29:247–255.
3. Chatterjee AS. The treatise on indian medicinal plants: Council of scientific and industrial research. New Delhi: Publication and Information Directorate; 1994. [Google Scholar]
4. Das K, Chakraborty PP, Ghosh D, Nandi DK. Protective effect of aqueous extract of *Terminalia arjuna* against dehydrating induced oxidative stress and uremia in male rat. *Iran J Pharma Res*,2010:9(2):153–161. [PMC free article] [PubMed] [Google Scholar]
5. Lukmanul H, Girija A, Boopathy R. Antioxidant property of selected *Ocimum* species and their secondary metabolite content. J Med Plants Res,2008:2(9):250–257. [Google Scholar]
6. Mandal A, Das K, Nandi D K. *In vitro* bioactivity study of bark extract of *Terminalia arjuna* on probiotics, commercially available probiotic formulation. *Int J Phytopharmacol*,2010:1(2):109–113.
7. Mojab F, Kamalinejad M, Ghaderi N, Vanidipour HR. Phytochemicals screening of some species of Iranian plants. Iran J Pharm Res,2003;3:77–82. [Google Scholar]
8. Nema R, jain P, Khare S, Pradhan A, Gupta A, Singh D. (2012). Antibacterial and antifungal activity of *Terminalia arjuna* leaves extract with special reference to flavanoids. *Basic Res J Med Clin Sci*,2012:1(5):63–65. [Google Scholar]
9. Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr J Biomed Res*,2007:10:175–181. [Google Scholar]
10. Parekh J, Chanda S. Phytochemicals screening of some plants from western region of India. Plant Arch,2008:8:657–662. [Google Scholar]
11. Pascaline J, Charles M, Lukhoba C, George O. Phytochemical constituents of some medicinal plants used by the Nandis of South Nandi district, Kenya. J Anim Plant Sci,2011;9:1201–1210. [Google Scholar]
12. Kharkar R *et al*., Antidiabetic activity of *Terminalia arjuna* (Roxb.) Wight & Arn. extract in alloxan induced diabetic rats, 2013.
13. John Wiley R, Sons. Medicinal plants and traditional medicine in Africa, 1993, 191–289. [PubMed] [Google Scholar]
14. Wang SY, Jiao H. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. J Agric Food Chem,2000:48(11):5672–5676.
15. Yadav RN, Agrawala M. Phytochemical analysis of some medicinal plants. J Physiol,2011:3(12):10–14.