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To study the antimicrobial and antioxidant potential of *Terminalia arjuna* leaves and bark extracts

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Abstract
Terminalia arjuna, a medicinal tree native to South Asia, has garnered attention for its broad pharmacological properties. This study focuses on the antimicrobial and antioxidant potential of extracts derived from the leaves and bark of *Terminalia arjuna*. The primary aim was to evaluate the bioactive properties of these extracts, utilizing different solvents to maximize the extraction of phenolic compounds, flavonoids, and other bioactive constituents. Both leaves and bark extracts were subjected to *in vitro* assays to assess their antimicrobial activity against a range of bacterial and fungal pathogens, including *B. subtilis*, *Escherichia coli*, *Candida albicans* etc. The antimicrobial efficacy was evaluated by broth dilution method for minimum inhibitory concentration (MIC). The antioxidant potential was investigated using assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity, ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assay, and total phenolic content estimation. The results revealed that both the leaves and bark extracts exhibited significant antimicrobial activity, with the leaves extract showed slightly higher potency, likely due to its higher phenolic content. Furthermore, both extracts demonstrated strong antioxidant activity, with % scavenging activity values comparable to standard antioxidants like ascorbic acid. The findings suggest that *Terminalia arjuna* extracts could serve as a natural source of antimicrobial and antioxidant agents, potentially useful for therapeutic applications in treating infections and combating oxidative stress-related disorders. Further studies are recommended to isolate specific bioactive compounds and explore their mechanisms of action.

Keywords: *Terminalia arjuna*, antimicrobial activity, antioxidant potential, leaves extract, bark extract, natural bioactive agents, DPPH assay, minimum inhibitory concentration (MIC)

Introduction
Medicinal plants have been at the core of traditional medicine systems across the world for centuries. These plants, rich in bioactive compounds, have been explored for their therapeutic potential to treat a variety of diseases and health conditions. Among the vast array of medicinal plants, *Terminalia arjuna*, commonly referred to as Arjuna, holds a prominent place in traditional Ayurvedic medicine. Indigenous to the Indian subcontinent, *Terminalia arjuna* belongs to the Combretaceae family and is a large deciduous tree, widely recognized for its therapeutic applications, especially in the treatment of cardiovascular diseases. The bark of *Terminalia arjuna* has been extensively used for treating heart ailments, while both the bark and leaves have gained recognition for their antioxidant and antimicrobial properties. Given the rising global concern regarding antimicrobial resistance and oxidative stress-related disorders, natural sources of antimicrobial and antioxidant agents, such as *Terminalia arjuna*, have attracted substantial scientific interest. The use of *Terminalia arjuna* dates back to ancient Indian medical texts, where it has been described as a potent cardiotonic agent. The bark, in particular, has been a cornerstone in Ayurvedic medicine for treating angina, heart failure, hypertension, and other cardiovascular issues. It has been traditionally prepared in various formulations, including decoctions, powders, and tinctures, to address these health concerns. Beyond its cardiovascular benefits, *Terminalia arjuna* has also been used in the treatment of asthma, ulcers, wounds, and inflammation [1]. Ethnobotanical surveys reveal that the use of *Terminalia arjuna* is not restricted to the Indian subcontinent; several Asian cultures have also integrated the plant into their healing practices. The rich polyphenolic content, particularly the flavonoids and tannins, contributes to its wide range of therapeutic properties. In addition to its well-documented

cardioprotective effects, recent studies have indicated that *Terminalia arjuna* may also possess significant antimicrobial and antioxidant potential, making it a valuable plant for further scientific exploration. Autoimmune disorders could potentially result from the infection and cause immune system problems [2]. Various resistant strains, such as MRSA (Methicillin-resistant Staphylococcus aureus), antibiotic-resistant *H. pylori*, strains of Streptococcus pyogenes resistant to erythromycin and tetracycline, and fluconazole-resistant *Candida albicans*, are posing significant challenges for researchers in the antimicrobial field [3]. Several studies have investigated the therapeutic potential of *Terminalia arjuna* in various contexts. The antimicrobial properties of *Terminalia arjuna* extracts have been explored against a wide range of bacterial and fungal pathogens. For instance, extracts from the bark and leaves have shown activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*, among others. These pathogens are responsible for many infections, and the ability of *Terminalia arjuna* to inhibit their growth highlights its potential as a natural antimicrobial agent [4]. Similarly, the antioxidant potential of *Terminalia arjuna* has been demonstrated in several studies. The bark, in particular, has been found to possess high antioxidant activity, which is thought to contribute to its cardioprotective effects. The antioxidant activity of *Terminalia arjuna* extracts has been evaluated using various assays, such as DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging, ABTS (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) scavenging, and FRAP (Ferric Reducing Antioxidant Power). These studies have confirmed that both the leaves and bark of *Terminalia arjuna* exhibit significant free radical scavenging activity, further supporting its potential as a natural antioxidant [7-8]. A wide range of scientific studies have been conducted on *T. arjuna* bark, including pharmacological activity studies, active constituent isolation and characterisation, quality assessment, and authenticity [9]. Moreover, a few clinical investigations on *T. arjuna* bark have been carried out16, 19. Conversely, hardly many research have been done on *T. arjuna* leaves. In a similar manner, the phytochemical compositions (including tannins, phenolics, and flavonoids) of the fruits, leaves, and bark of *T. arjuna* [10] were assessed both qualitatively and quantitatively to establish a relationship between the phytochemical content and their biological activities.

This study seeks to address these gaps by systematically investigating the antimicrobial and antioxidant potential of methanolic extracts of *Terminalia arjuna* leaves, bark and fruits. By doing so, it aims to contribute to the growing body of research on the medicinal properties of this plant, while also offering potential solutions to the global challenges of antibiotic resistance and oxidative stress.

Materials and Methods

Materials: Methanol, acetone, chloroform etc. were purchased from S.D. Fine chemicals, Mumbai. DPPH, MTT powder, DMSO were obtained from Sigma Aldrich. All other chemicals and reagents were used for antimicrobial and antioxidant experiments were obtained from Hi-Media, Mumbai.

Collection, processing and extraction of bark and leaves: After proper identification, the freshly plant material was collected for research study from Shahapur region, Dist-Thane, Maharashtra, India. The collected plant material i.e. fruits, bark and leaves were washed thoroughly with running water 2 to 3 times and dried in a cool place for 2 to 3 days at room temperature. Once completely dried, the plant materials were grinded into powder and stored in a small plastic bottle with suitable paper.

Fifty grams of dried powder made from the bark and leaves of *T. arjuna* were combined with 500 ml of methanol in a conical flask, which was sealed with cotton wool. The mixture was then placed on a rotary shaker set at 120 rpm and allowed to incubate for 5 days to ensure thorough extraction [11]. The extracts were passed through Whatman No. 1 filter paper and then centrifuged at 4000 g for 5 minutes. The solvent layer was gathered and evaporated at 40 °C. The resulting dried crude extracts were kept in airtight bottles at 4 °C until needed. This extraction process was repeated three times to guarantee thorough extraction. The crude methanolic extract was then dissolved in distilled water, followed by successive fractionation using chloroform, ethyl acetate, n-butanol, and the remaining aqueous fraction [12].

Qualitative analysis of phytochemicals

The methanolic extract and its different fractions (chloroform, ethyl acetate, n-butanol, and aqueous) from the bark and leaves of *T. arjuna* were analyzed using various chemical tests to identify several phytocomponents, including phenolics, tannins, flavonoids, phytosteroids, and saponins, following established protocols [13].

Quantitative Analysis of Phytochemicals

Determination of Total Phenolic Content (TPC)

The total phenolic content (TPC) in FME, LME, and BME was evaluated using a spectrophotometric method known as the Folin-Ciocalteu method, as outlined by *Singleton et al.* The findings were determined based on a gallic acid standard curve and expressed in terms of gallic acid equivalents (GAE).

Determination of Total Flavonoid Content (TFC)

The total flavonoid content (TFC) for each extract was determined using the aluminum chloride colorimetric method described by Zhishen *et al*. [14]. Flavonoids levels were determined using a quercetin calibration curve and expressed as quercetin equivalents (QE).

Determination of Condensed Tannin Content (CTC)

The vanillin assay outlined by Sun *et al*. 2004 was utilized to assess CTC levels in FME, LME, and BME. CTC was measured using a catechin standard curve and reported as catechin equivalents (CE).

***In vitro* Evaluation of Antioxidant Activity**

Different antioxidant methods were utilized to assess the antioxidant potential of FME, LME, and BME. Ascorbic acid and BHT (Butylated hydroxytoluene) served as standard references in all the tests.

DPPH Free Radical Scavenging Assay

The free radical scavenging activity of each extract was evaluated using DPPH (2, 2-diphenyl-1-trinitrophenylhydrazine) [15]. The difference between the extracts and ascorbic acid (as standard) was prepared using

methanol (Sigma-Aldrich) as the solvent. Approximately 1 mL of each concentration was placed in a test tube and 0.5 mL of 1 mmol/L DPPH in methanol was added. The tubes were then incubated for 15 min and the absorbance was measured at 517 nm. A white solution was prepared using DPPH dissolved in the same volume of methanol [16]. Calculate the percent inhibition (PI) of DPPH radicals using the following formula:

PI = $AC-AE/AC$ x 100

Where AC= absorbance of the control (DPPH + methanol) and AE = absorbance of the extract. The IC50 value denotes the extract concentration necessary to scavenge DPPH free radicals by 50%.

Hydrogen Peroxide (H2O2) Scavenging Assay

The ability of each extract to remove H2O2 was evaluated. Phenol (12 mM) and 4-aminoantipyrene (0.5 mM) were chosen for all experiments because these concentrations resulted in the highest chromophore intensity. Determine the percent inhibition (%I) of H_2O_2 by herbal extracts and standard antioxidants using the formula [17].

H_2O_2 scavenging (%) = $AC-AEAC \times 100$

Where AC= Absorbance of the control (H_2O_2 + methanol) and AE= absorbance of the extract. The IC50 value represents the extract concentration required to scavenge H2O2 by 50%.

Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP test was used to measure the reducing power of the extracts. During this process, ferricyanide takes electrons from the antioxidant and turns into the reduced form of ferrocyanide. Therefore, in the presence of ferric chloride, the color changes from yellow to Prussian blue. Unlike the determination of DPPH and H2O2, this method is based on the principle that an increase in the absorbance of the reaction mixture indicates a higher redox potential of the extract [18].

Total Antioxidant Capacity (TAC) Assay

The TAC (Phosphomolybdenum) test was originally developed to measure vitamin E in seeds, but its use has been expanded to include plant extracts because of its ease of use and sensitivity. In this test, molybdenum (VI) is reduced to molybdenum (V) by electron transfer or hydrogen atom transfer. As the reaction occurs, the color changes from yellow to blue-green, indicating the strong antioxidant activity of the extract, which is reflected in higher absorbance readings? A significant advantage of the TAC test is its ability to measure a variety of samples, including lipophilic plant extracts. However, this test has poor correlation with flavonoid and phenolic content, as it can detect other substances such as ascorbic acid, carotenoids, and alpha-tocopherol.

Evaluation of Antimicrobial Activity

Based on preliminary experiment and literature study, the ethanolic extract of *Terminalia arjuna* bark and leaves was tested for its antimicrobial properties using different concentrations against gram-positive bacteria (*Bacillus subtilis*), gram-negative bacteria (*Escherichia coli*), and yeast (*Candida albican)*. The extracts demonstrated varying levels of antimicrobial effectiveness against the tested microorganisms. The findings were reported based on observations made at 24hours of incubation [20].

Assay of Antibacterial activity using agar well diffusion method

Measure Gram-positive bacteria (*Bacillus subtilis*), Gram-negative bacteria (*E. coli*) and yeast (*Candida albican*) using the agar diffusion method using various dilutions concentrations to measure antibacterial and antifungal properties. All cultures were maintained on nutrient agar slopes/plates and incubated at 30-25 °C for 24 to 48 hours. Every 24 hours, pure culture was transferred to a Roux bottle containing 5 ml of saline and stored appropriately. Solvent-free extracts were used as control experiments [21-22].

A. Preparation of broth medium: 1.3 gm nutrient broth was dissolved in 100 ml of distilled water and sterilized at 100°C for 20 min in autoclave. After cooling the medium pour it in 96 well plates in quantity of 100 µl in each well.

B. Inoculum Preparation

Growth Method

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at 35 °C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (Usually 2 to 6 hours)
3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. This results in a suspension containing approximately 1 to 2×10^8 CFU/ml for microorganisms under study. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

C. Preparation of samples: Stock solutions of the plant extracts and the positive control drug streptomycin were prepared in dimethyl sulphoxide (DMSO) at the concentrations of 10 mM/ml.

D. Preparation of plates: The minimum inhibitory concentration (MIC) of the extracts for each test organism was determined by a modification of the broth dilution method performed in 96 well micro-trays (NCCLS, 1993). Two-fold serial dilutions of the compounds (150 µl) of each sample were made in sterile broth (Nutrient broth) to achieve a concentration range of 0.01-100 µl/ml. The amount of test organisms (50 µl) was added to each dilution to give a final volume of 200 µl. After incubation at 37 °C for 18–24 h the plates were examined for growth of the organisms. The lowest concentration that inhibited growth was recorded as the MIC. Growth was determined as the difference in absorbance at 595nm between reading taken before and after incubation of the plates. Readings less than 20% of the positive control were recorded as inhibition. Absorbance was read in a plate reader. Four replicates were performed for compounds against each of the test organisms. Positive (100 µl cells + 100 µl medium) and negative (200 µl medium). Controls were performed with

each experiment. Each plate also included a solvent control in which 100 µl of the appropriate diluted solvent was added to 100 µl of the test organism.

E. Data analysis: The data analysis was accomplished using Graph Pad InStat version 6.01 for Windows 95, GraphPad Software Inc., San Diego California USA. IC50 values were obtained from regression lines with coefficient factors between R2 = 0.52 and 0.99. Absorbance at 595nm between reading taken before and after incubation of the plates.

Assay of Antifungal Activity

Antifungal susceptibility test using standard method of NCCLS (The National Committee for Clinical Laboratory Standards)

a) Preparation of broth medium: 10.4 gms of RPMI-1640 medium supplemented with glutamine and phenol red, without bicarbonate and 34.53g 3-(N-morpholino) propanesulfonic acid (MOPS) was dissolved in 400 ml of distilled water. pH was adjusted to 7.0 at 25 °C with 1 mol/L sodium hydroxide. The volume was made up to 0.5 L with water and was filtered, sterilized and was stored at 4°C until required.

b) Preparation of inocula: Fungal strain was sub-cultured on to their respective growth medium and incubated for 48 hrs at 25-30 °C. From these plates, several colonies were transferred to 5 ml of sterile distilled water. The suspensions were mixed for 15 s to ensure homogeneity and were subsequently diluted to match the turbidity of a 0.5 McFarland standard (i.e. OD = 0.12–0.15 at k = 530 nm, corresponding to 1–5* 10^6CFU/ml). Then the working suspensions were prepared by a 1 in 30 further dilution of the stock suspension in sterile distilled water to yield 1–5 * 10^3 CFU/ml. 0.1ml sterilized solution of resazurin (20 mg/ml in water) was supplemented to the working suspension.

c) Preparation of samples: Stock solutions of the sample extracts were taken as such with 1000µl/ml and the positive control drug Amphotericin B were prepared in dimethyl sulphoxide (DMSO) at the concentrations of 100 mg/ml. Further it was diluted to 1:50 in broth.

d) Preparation of plates: Microdilution susceptibility testing was performed in flat bottom 96-well clear plates containing broth medium (50µl) in each well. Sample solutions (50µl) were subsequently serially diluted two-fold in the plates with the broth, starting with the final concentration of 5000 mg/L. The working inoculum suspension (50µl) was added to give a final inoculum concentration of 0.5–2.5*10^3 CFU/ml. Fluconazole was used as the standard antifungal drug. Sterility and growth controls in the presence of DMSO were also included. The plates were then incubated at 37 °C for 48h. The amount of growth was measured using plate reader at λ=492 nm, [NCCLS document M27-A2] Percentage inhibition of the extract against all cell line was calculated using the following formula.

% Cell survival = (AT-AB) / (AC-AB) X 100

Where,
AT=Absorbance of treated cells (drug)
AB=Absorbance of blank (without cell)
AC=Absorbance of control (untreated)
There by,
% Cell growth inhibition= = 100-% cell survival

Result & Discussion

Table 1: Percentage cell inhibition by test compounds against selectedstrain of bacteria.

Conc (µg/ml)	Log Conc.	Bark extract		Leaves extract		STD	
		E. coli	*B. subtilis*	*E. coli*	*B. subtilis*	*B. subtilis*	*E. coli*
0.01	-2.29	5.972	0.36	8.66	-0.12	9.88	8.69
0.02	-1.82	10.78	0.42	10.28	7.498	11.88	10.32
0.05	-1.34	9.4875	1.62	12.59	13.22	14.03	12.69
0.14	-0.86	10.998	5.87	15.72	21.87	17.96	15.67
0.41	-0.39	14.324	5.96	29.35	22.68	29.12	29.62
1.23	0.09	17.750	6.88	31.78	27.26	33.24	31.87
3.70	0.57	20.702	8.021	38.52	31.55	41.87	38.49
11.11	1.05	28.667	10.67	59.22	60.02	48.67	59.03
33.33	1.52	34.652	13.22	65.24	68.71	69.87	65.34
100.00	2.00	67.523	39.062	91.24	88.63	85.37	91.08
IC50 µl/ml		91.73	>100	7.254	8.437	8.362	11.55
R^2		0.9522	0.9436	0.9445	0.9461	0.9449	0.8564

Table 2: Percentage growth inhibition of Leaves extract against fungal strains and Fluconazole (Std.)

Conc (µg/ml)	Log Conc.	% Cell Inhibition			
		C. albican	*C. fumigatus*	*A. niger*	STD
0.05	-1.29	0.52	0.452	0.24	2.687
0.15	-0.82	8.42	0.983	11.35	7.321
0.46	-0.34	17.35	1.879	15.06	11.52
1.37	0.14	30.05	7.362	31.88	16.08
4.12	0.61	35.04	14.862	42.03	33.65
12.35	1.09	46.35	25.571	49.73	49.21
37.04	1.57	58.92	37.241	66.89	68.05
111.11	2.05	69.21	48.623	73.02	71.08
333.33	2.52	79.26	52.421	77.98	81.22
1000	3.00	87.06	81.320	88.62	96.57
IC_{50} µl/ml		9.562	38.65	5.328	10.30
R^2		0.9496	0.9397	0.9588	0.9767

Table 3: Qualitative analysis of phytoconstituents present in *T. arjuna* FME, LME and BME

Type of content	FME	BME	LME
Saponins	+	+	+
Antraquinones	-	-	-
Alkaloids	+	+	+
Flavonoids	+	+	+
Carbohydrate & Glycosides	+	+	+
Terpenoids	+	+	+
Polyphenolics	+	+	+

FME: Fruit methanolic extract, BME-Bark Methanolic extract, LME-Leaves Methanolic Extract

Table 4: Antioxidant study assay of *T. arjuna*

Sample	DPPHIC50 (µg/mL)	H_2O_2 IC50 (µg/mL)	FRAP (mMol Fe+2/g)	TAC mg GAE/g
FME	16.9±1.36	30±2.17	1.94±0.62	60.92±3.96
LME	12.2±1.12	20.6±1.86	3.21±0.65	61.74±3.42
BME	13.3±1.07	33.1±1.94	2.54±0.48	53.27±4.39
Ascorbic acid	10.6±0.8	12.74±0.86	2.93±0.75	124.83±7.21
BHT	8.24±0.62	12.3±0.7	6.84±0.92	76.91±4.53

FME: Fruit Methanolic extract, BME-Bark Methanolic extract, LME-Leaves Methanolic Extract

Table 5: Percentage scavenging activity of *T. arjuna*

Conc. (µg/mL)	H2O2 Scavenging%			DPPH scavenging %		
	FME	LME	BME	FME	LME	BME
1000	93.26	96.32	92.68	95.63	98.32	95.26
500	90.63	92.35	87.96	94.22	95.63	94.32
250	88.22	90.35	85.62	92.61	93.22	91.20
125	82.63	88.32	80.93	91.74	92.34	90.12
62.5	72.88	85.31	70.25	88.36	91.78	88.36
31.25	59.32	71.45	55.37	80.24	92.34	88.47
15.62	41.53	50.36	36.82	61.34	74.25	59.31
7.81	20.43	30.22	21.57	22.31	44.36	46.88
3.90	10.75	25.03	7.38	12.31	30.24	30.62
1.95	5.22	11.75	5.03	5.02	24.33	19.02
0.97	0.82	0.52	0.89	0.56	0.52	0.34

FME: Fruit Methanolic extract, BME-Bark Methanolic extract, LME-Leaves Methanolic Extract

Conclusions

The study revealed that certain antimicrobial phytochemical found in the methanolic extracts of *Terminalia arjuna* inhibit the growth and physiological processes of bacteria like *Candida albican*, *Bacillus subtilis* and *Escherichia coli*. The methanolic extract of leaves showed moderate to high antimicrobial activity against both the Gram-positive *Bacillus subtilis* and the Gram-negative *Escherichia coli* bacteria. The study also examined the antioxidant and free Radical scavenging activities characteristics of *T. arjuna* fruits, leaves, and bark; it was discovered that the leaf extract had the strongest antimicrobial activity against *Bacillus subtilis* and the highest scavenging activities against DPPH free radicals and hydrogen peroxide. *T. arjuna* leaf methanolic extract exhibits antifungal activity against the fungus species like *Aspergillus niger* and *Candida albican*. The study revealed that with a broader antimicrobial activity demonstrating its application in various herbal formulations.

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References

1. Khatkar S, Nanda A, Ansari SH. Improved methods of extraction and *in vitro* evaluation of antimicrobial potential of stem bark of *Terminalia arjuna*. Curr Biochem Eng. 2019;5(1):50-56. doi:10.2174/2212711906666190131151937.
2. Fahey JW, *et al*. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzoapyrene-induced stomach tumors. [Online] Available: www.pnas.org/cgi/doi/10.1073/pnas.112203099.
3. Morschhäuser J. The development of fluconazole resistance in *Candida albicans*: An example of microevolution of a fungal pathogen. Microbiol. Soc. Korea. 2016 Mar; doi:10.1007/s12275-016-5628-4.
4. Rufino M do S, Alves RE, de Brito ES, Pérez-Jiménez J, Saura-Calixto F, Mancini-Filho J. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil. Food Chem. 2010;121(4):996–1002. doi:10.1016/j.foodchem.2010.01.037.
5. Jain S, Yadav PP, Gill V, Vasudeva N, Singla N. *Terminalia arjuna*: A sacred medicinal plant: phytochemical and pharmacological profile. Phytochem

Rev. 2009;8(2):491-502. doi:10.1007/s11101-009-9134-8.
6. Suganthy N, Muniasamy S, Archunan G. Safety assessment of methanolic extract of Terminalia chebula fruit, *Terminalia arjuna* bark and its bioactive constituent 7-methyl gallic acid: *in vitro* and *in vivo* studies. Regul Toxicol Pharmacol. 2018;92:347–357. doi:10.1016/j.yrtph.2017.12.019.
7. Kapoor D, Vijayvergiya R, Dhawan V. *Terminalia arjuna* in coronary artery disease: ethnopharmacology, pre-clinical, clinical & safety evaluation. J Ethnopharmacol. 2014 Sep 11. doi:10.1016/j.jep.2014.06.056.
8. Sandhu J, Shah B, Shenoy S, Chauhan S, Lavekar G, Padhi M. Effects of Withania somnifera (Ashwagandha) and *Terminalia arjuna* (Arjuna) on physical performance and cardiorespiratory endurance in healthy young adults. Int J Ayurveda Res. 2010;1(3):144. doi:10.4103/0974-7788.72485.
9. Amalraj A, Gopi S. Medicinal properties of *Terminalia arjuna* (Roxb.) Wight & Arn.: A review. J Tradit Complement Med. 2017 Jan; doi:10.1016/j.jtcme.2016.02.003.
10. Mehmood N, Zubair M, Rizwan K, Rasool N, Shahid M, Ahmad VU. Antioxidant, antimicrobial and phytochemical analysis of Cichorium intybus seeds extract and various organic fractions. Int J Med Chem. 2012;2012:1–6. doi:10.1155/2012/157125.
11. Kumar V, *et al*. Comparative analysis of phytochemicals, antimicrobial and antioxidant activity of different species of Terminalia from Himachal Pradesh, India. Vegetos. 2021;34(3):528-539. doi:10.1007/s42535-021-00232-y.
12. Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1999 Mar;64(4):555-559.
13. Yen G-C, Duh P-D. Scavenging effect of methanolic extracts of peanut hulls on free-radical and active-oxygen species. Food Chem; c1994.
14. Barros L, Falcão S, Baptista P, Freire C, Vilas-Boas M, Ferreira ICRF. Antioxidant activity of Agaricus sp. mushrooms by chemical, biochemical and electrochemical assays. Food Chem. 2008;111(1):61-66. doi:10.1016/j.foodchem.2008.03.033.
15. Fernando CD, Soysa P. Optimized enzymatic colorimetric assay for determination of hydrogen peroxide (H2O2) scavenging activity of plant extracts. MethodsX. 2015;2:283-291. doi:10.1016/j.mex.2015.05.001.
16. Irshad M, Zafaryab M, Singh M, Rizvi MMA. Comparative analysis of the antioxidant activity of Cassia fistula extracts. Int J Med Chem. 2012;2012:1–6. doi:10.1155/2012/157125.
17. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999. [Online] Available: http://www.idealibrary.com.
18. Bark A, Prajapati P, Mehta P, Prajapati M. Antimicrobial activity of ethanolic extract of *Terminalia arjuna* (Roxb.). 2021. [Online] Available: http://ijmer.in/pdf/e-Certificate%20of%20Publication-IJMER.pdf.
19. Subramanian R, *et al*. Antimicrobial activity of aqueous extracts of bark, root, leaves and fruits of *Terminalia arjuna* Wight & Arn. 2008. [Online] Available: https://www.researchgate.net/publication/235919102.
20. Valsaraj R, Smitt U, Adsersen A, Nyman U. Antimicrobial screening of selected medicinal plants from India. 1997.
21. Mendonça RD, Pimenta AM, Gea A, de la Fuente-Arrillaga C, Martinez-Gonzalez MA, Lopes AC, *et al*. Ultraprocessed food consumption and risk of overweight and obesity: the University of Navarra Follow-Up (SUN) cohort study. Am J Clin Nutr. 2016 Nov;104(5):1433–40. doi:10.3945/ajcn.116.135004. Epub 2016 Oct 12. PMID: 27733404.