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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF *Terminalia arjuna* BARK EXTRACT

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ABSTRACT

Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. In the present study aimed to investigate the phytochemical and antimicrobial activity of *Terminalia arjuna* bark extract. The study demonstrated that the preliminary phytochemical analysis of aqueous and hydro alcoholic extract of *Terminalia arjuna* bark revealed the presence of flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids and phlobatannins, Phlobatannins and anthroquinones. Significant quantity of phytochemicals present in Terminalia arjuna bark. The phytochemicals further confirmed in TLC. The fluorescence behaviors of plant powder were observed. Terminalia arjuna bark possess antibacterial activity against *E. Coli*. Overall, the *Terminalia arjuna* bark is a source of phytochemicals and possesses antimicrobial activity that can be important in health prevention.

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INTRODUCTION

In wide-ranging dietary phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources (Moorachian, 2000). Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, bark, bark, fruits or seeds (Costa *et al.*, 1999). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions(King and Young,1999). Phytochemicals are also available in supplementary forms, but evidence is lacking that they provide the same health benefits as dietary phytochemicals.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can

protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc. In the present study aimed to investigate the phytochemical and antimicrobial activity of *Terminalia arjuna* bark extract.

MATERIALS AND METHODS

Collection of plant materials

The Terminalia arjuna bark were collected from Thanjavur on April 2016. The collected plant parts were cut into small pieces and shade dried at room temperature and makes a fine powder using grinder mixture.

Preparation of extract

The powder was extracted with 100% ethanol, methanol, aqueous and 70% methanol for 24 hours soaking. After 24 hours a semi solid extract was obtained after complete elimination of alcohol/water under reduced pressure. The extract was stored in refrigerator until used.

Qualitative Analysis

Preliminary phytochemicals screening: Chemical tests were carried out on the alcoholic extract using standard procedures to identify the preliminary phytochemical screening following the methodology of Sofowara (1993), Trease and Evans (1989) and Harborne (1973).

Quantitative Analysis

Determination of total phenols by spectrophotometric method. Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994). Alkaloid determine by the method of Harborne (1973). Total terpenoid content in the leaf extracts were assessed by standard method (Ferguson, 1956).

Qualitative Analysis of Vitamins

Vitamin- A,C, D and Vitamin -E test carried out by Pearson, (1976) and Patel, (2005).

Fluorescence Behavior

The fluorescence studies were carried out as per the method of Bhattacharya and Zaman (2009).

Determination of Antibacterial Activity

Antibiogram was done by disc diffusion method (NCCLS, 1993; Awoyinka *et al*., 2007) using plant extracts. Petri plates were prepared by pouring 30 ml of NA medium for bacteria. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria from a broth culture. A sterile cotton swab is dipped into a standardized bacterial test suspension and used to evenly inoculate the entire surface of the Nutrient agar plate. Briefly, inoculums containing of Escherichia coli specie of bacteria were spread on Nutrient agar plates for bacteria. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude sample (50µl, 100µl and 150µl) was laid down on the surface of inoculated agar plate. The plates were incubated at 37○C for 24 h for the bacteria and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate.

Measurement of zone of inhibition

The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the extracts were measured using a millimeter scale.

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *Terminalia arjuna* bark revealed the presence of chemical constituents like Tannin, Saponin, Terepenoids, Carbohydrate, and Glycoside (Table 1) in maceration and percolation extract which was confirmed by performing TLC separation technique and different spraying reagents. R_f values were calculated.

Thin-layer chromatography

Thin-layer chromatography (TLC) is still being used as separation tools for many phytochemicals due to the convenience, low cost, simultaneous separation and detection of considerable amount of samples and the availability of new stationary phases. The crude extracts of plant materials contain highly complex profiles of phytochemical and often, isocratic separation cannot achieve satisfactory separation. Multiple mobile phases, in regular or TLC are therefore useful for good separation of phytochemicals from complicated plant materials or extracts. TLC is also one of the main methods for class fractionation and speciation of lipids and is used increasingly to determine the botanical origin, potency, and flavour potential of plant materials (e.g. herbs and spices). The majority of the TLC applications are in the fractionation and preliminary separation of phytochemicals before they are separated, quantified and identified by HPLC or other high-performance separation techniques (Tsaoa *et al*., 2004).

Many core and new TLC technologies have been identified and developed in recent years, including: (1) methods to provide a constant and optimum mobile phase velocity (forced flow and electroosmotically-driven flow), (2) video densitometry for recording multidimensional chromatograms, (3) in situ scanning mass spectrometry, and (4) bioactivity monitoring for selective detection. These technologies, in combination with 2D, multiple development and coupled column–layer separation techniques could dramatically increase the use of TLC for the characterization of complex mixtures such as plant

extracts containing phytochemicals (Lukasz and Monika, 2009).

Vitamins

Vitamins are organic substances that are essential in tiny amounts for growth and activity of the body. They are obtained naturally from plant and animal foods. Organic in this definition refers to the chemistry and molecules of vitamins. The word organic means that the molecules of the substance contain the element carbon. The term also means that vitamins can be destroyed and become unable to perform their functions in our bodies. Too much heat, certain kinds of light and even oxygen can destroy some vitamins. The amounts of vitamins ingested from food are measured in micrograms or milligrams (Okwu, 2004).

Table 1: Phytochemical screening of *Terminalia arjuna* bark

S.No	Phytochemical analysis	Methanol 100%	Ethanol 100%	Water 100%	Hydro-alcohol 100%
1	Tannin	+	_	+++	++
2	Phlobatannins	+	_	++	++
3	Saponin	++	+	++	++
4	Flavonoids	+	+	+	++
5	Steroids	+++	_	++	+++
6	Terepenoids	++	_	++	+
7	Triterpenoids	++	_	++	+
8	Alkaloids	++	_	++	+
9	Carbohydrate	++	+	+++	++
10	Protein	+	+	++	+
11	Anthroquinone	++	+	++	+
12	Polyphenol	+	_	+++	+
13	Glycoside	_	_	+++	++

+ = Presence; - Absence; (++ = Moderately present)

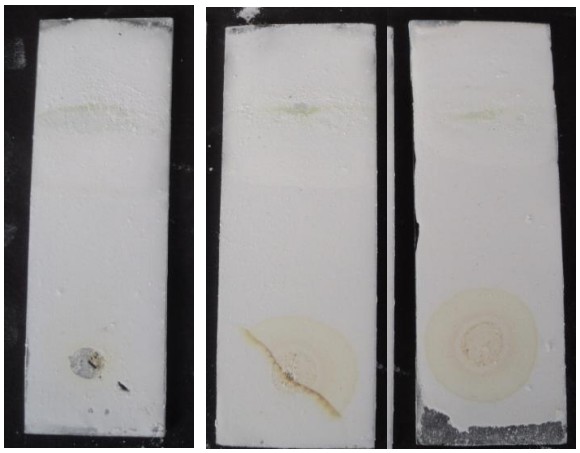
Table 2: Quantitative analysis of *Terminalia arjuna* bark

Secondary metabolites	Result (mg/gm)
Phenol	0.880
Flavonoids	0.213
Alkaloids	0.128
Terepenoids	0.199

Table 3: Thin layer chromatographic separation of compounds in *Terminalia arjuna* bark

S. No	Name of the test	Rf Values
1	Flavonoids	0.51
2	Phenols	0.73
3	Terepenoids	0.65

Fig 1: Thin layer chromatographic separation of compounds in *Terminalia arjuna* bark



Flavonoids **Phenols** **Terepenoids**

Vitamin D is important in bone formation. Most vitamin D is made when sunshine hits the skin. Too much sun can contribute to skin cancer, and using a sunscreen of SPF 15 or more will block vitamin D formation. Milk and margarine are both fortified with vitamin D. Those over the age of 65 only make about half as much vitamin D as children from the same amount of light exposure, so it is recommended to take a supplement for these people to get enough vitamin D. A vitamin D deficiency can cause an older disease called rickets, and it is cured by cod-liver-oil, which has a high concentration of vitamin D. Vitamin D is stored in the liver and as little as 5 times the Daily Value can produce unhealthy weight loss, vomiting, and calcium deposits in the lungs and kidneys (Clark, 2008). The vitamins of the *Terminalia arjuna* bark investigated and summarized in Table-4.

Table 4: Qualitative analysis of vitamins in *Terminalia arjuna* bark

S.ON	Vitamins	Observation
1	Vitamin A	--
2	Vitamin C	++
3	Vitamin D	+
4	Vitamin E	++

+ = Presence; - Absence; (++ = Moderately present)

Fluorescence analysis

The fluorescence analysis is a tool for the determination of constituents in the plant that gives a definite idea of the chemical nature. Thus the fluorescence analysis of the powder was carried out and data is presented in the Table 5. Similarly extracts were also subjected to UV chamber and fluorescence was observed and consistency was noted as an additional character for identification. Fluorescence analysis of the powdered drugs were performed and tabulated which helps to detect the adulteration, because phyto constituents exhibits characteristic fluorescence under ultraviolet light when they got mixed with the reagents. The fluorescence exhibited by the mixture was attributed to the chemical constituents present in the crude drug. Prior to the phyto chemical screening a rough estimation of phyto constituents was done by the behaviour of powder drug with different chemical reagents which powdered drug showed different colours when it got mixed the particular reagents which reflects the presence phytochemicals in accordance with the colours obtained. Fluorescence behavior of *Terminalia arjuna bark* powder was represented in table 5.

Table 5: Fluorescence behaviour of Terminalia arjuna bark

S.No	Tests	Visible Light	Short UV 245	Long UV 366
1	Plant powder	Green	Green	Black
2	Plant powder treated with distilled water	Light green	Light green	Black
3	Plant powder treated with hexane	Yellow	Light green	Brown
4	Plant powder treated with chloroform	Light green	Yellow	Brown
5	Plant powder treated with methanol	Dark green	Dark green	Black
6	Plant powder treated with acetone	Light green	Light green	Blue
7	Plant powder treated with 1N sodium hydroxide in water	Yellow	Yellow	Yellow

8	Plant powder treated with 1N hydrochloric acid	Brown	Dark green	Yellow
9	Plant powder treated with sulphuric acid with an equal volume of water	Brown	Dark green	Yellow
10	Plant powder treated with nitric acid diluted with an equal volume of water	Brown	Light green	Brown

Antimicrobial activity

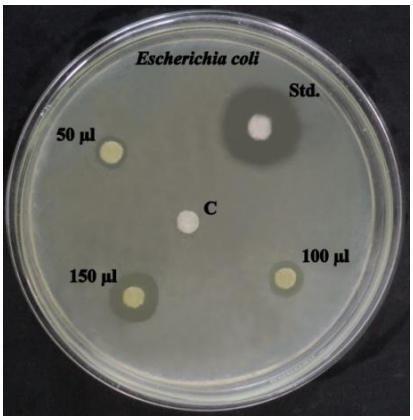
The *in vitro* antimicrobial activity of the *Terminalia arjuna* extract against these bacteria were qualitatively assessed by the presence of inhibition zones represented in the photographic Fig 2**.** The inhibitory activities in culture media of the *Terminalia arjuna* reported in Table 6 were comparable with standard antimicrobiotic viz. chloromphenical. The *E.coli* which already known to be multi-resistant to antibiotics, were resistant to tested plant extract. The mean inhibition zone of *Terminalia arjuna* extract was 2.40±0.16mm for 50 µl, 4.70±0.32mm for 100 µl, 7.80±0.54mm for 150 µl11.10±0.77 for standard for *E.coli*.

Table 6 Antibacterial activity of *Terminalia arjuna* bark

Microorganism	50 µl	100 µl	150 µl	Std.	control
Escherichia coli	2.40±0.16	4.70±0.32	7.80±0.54	11.10±0.77	0

Values were expressed as Mean ± SD.
Bacterial standard - Chloromphenical

Fig 2: Antibacterial (*Escherichia coli*) activity of *Terminalia arjuna* bark



CONCLUSION

Phytochemical simply means plant chemicals. "Phyto" is the Greek word for plant. Phytochemicals are classified as primary or secondary constituents, depending on their role in plant metabolism. The study demonstrated that the preliminary phytochemical analysis of aqueous and hydro alcoholic extract *Terminalia arjuna* bark revealed the presence of flavonoids, phenolics, steroids, tannin, saponins, glycosides, terpenoids and phlobatannins, Phlobatannins and anthroquinones. Significant quantity of phytochemicals present in *Terminalia arjuna* bark. The phytochemicals further confirmed in TLC. The fluorescence behaviors of plant powder were observed. *Terminalia arjuna* bark possess antibacterial activity against *E. Coli*. Overall, the *Terminalia arjuna* bark is a source of phytochemicals and possesses antimicrobial activity that can be important in health prevention.

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