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Analysis of phytochemical profile of *Terminalia arjuna* bark extract with antioxidative and antimicrobial properties

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PEER REVIEW

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Comments

The aim of the study is to investigate phytochemical screening, antimicrobial activity and qualitative thin layer chromatographic separation of flavonoid components, antioxidant activity and total flavonoid compound of *T. arjuna*. The *T. arjuna* bark extract revealed the presence of bio-active constituents which are known to exhibit medicinal as well as physiological activities.
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ABSTRACT

Objective: To investigate phytochemical screening, antimicrobial activity and qualitative thin layer chromatographic separation of flavonoid components, antioxidant activity and total flavonoid compound of *Terminalia arjuna*.
Methods: For phytochemical screening, some common and available standard tests were done. Antimicrobial bioassay was done through agar well diffusion method. Detection of antioxidant activity and flavonoid compounds were done through thin layer chromatography. Total antioxidant activity was measured by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) in colorimetric method. Aluminum chloride colorimetric method was used for total flavonoid determination.
Results: Phytochemical screening showed the active compounds presence in high concentration, such as phytosterol, lactones, flavonoids, phenolic compounds and tannins and glycosides. The antimicrobial activity of extract showed that greater inhibition zone against Gram negative bacteria than Gram positive bacteria. This methanolic extract showed a promising antioxidant activity, as absorption of DPPH redicles decreased in DPPH free radical scavenging assay. Flavonoids components having antioxidant property present in the methanol extract at a level of 199.00 mg quercetin equivalent/g of dried methanol extract in colorimetric method.
Conclusions: The *Terminalia arjuna* bark extract revealed the presence of bio-active constituents which are known to exhibit medicinal as well as physiological activities.

KEYWORDS
Terminalia arjuna, Phytocompound, TLC, Antioxidant, Total flavonoid, Antimicrobial activity, DPPH

1. Introduction

In the ancient India, medicinal plants were used to prevent various critical diseases. The plant kingdom is an important source of herbal drugs. Even in recent years, there has been an increasing awareness about the importance of medicinal plants. Generally, herbal drugs are easily available, safe, less expensive, efficient, and rarely have side effects. According to World Health Organization, medicinal plants would be the best source to obtain variety of drugs[1]. 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, flavonoids and phenols.

The bio-active phytocompounds are synthesized by primary or rather secondary metabolism of living organisms. Secondary metabolites are chemically and taxonomically extremely diverse compounds with obscure function. They are widely used in the human therapy,

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veterinary, agriculture, scientific research and countless other areas[2]. Medicinal plants containing active chemical constituents with high antioxidant property play an important role in the prevention of various degenerative diseases[3] and have possible benefits to the humanity. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effects on all types of microorganisms *in vitro*. Botanical medicines or phytomedicines refer to the use of seeds, berries, leaves, bark, root or flowers of any plant for medicinal purposes by significant number of people. Knowledge of the chemical constituents of plants is desirable because such information will be value for synthesis of complex chemical substances[4-6].

Terminalia arjuna (*T. arjuna*) is a deciduous large-sized fluted tree to 30 m tall and 2-2.5 m diameter at breast height, with an often buttressed trunk. *T. arjuna* (family Combretaceae), a large tree, is found throughout the South Asian region. This tree is usually an evergreen tree with new leaves appearing in the hot season (February to April) before leaf fall. This tree is an exotic tree in India. It is one of the most versatile medicinal plants having a wide spectrum of biological activity. The bark of *T. arjuna* is anti-dysentric, antipyretic, astringent, cardiotonic, lithotriptic, anticoagulant, hypolipidemi, antimicrobial[7] and antiuremic[8] agent. Many useful phytoconstituents have been isolated from *T. arjuna* which included triterpenoids for cardiovascular properties, tannins and flavonoids for its anticancer, antimicrobial properties and so on[9]. The powder of the bark acts as a diuretic in cirrhosis of liver and gives relief in symptomatic hypertension[10]. In studies in mice, its leaves have been shown to have analgesic and anti-inflammatory properties[11]. The purpose of the study was to find out the preliminary phytochemical screening of the extract and to determine the antioxidant and antimicrobial activity of extract of the bark of *T. arjuna*.

2. Methods and materials

2.1. Plant material collection

The bark of *T. arjuna* was collected from University Road, Vidyasagar University, Midnapore, Paschim Medinipur, district of West Bengal. The material was identified by the taxonomist of the Botany Department at the Raja N. L. Khan Women's College, Midnapore. The voucher specimens were deposited in the Department of Botany, Raja N. L. Khan Women's College.

2.2. Bacterial strain and culture conditions

Two Gram negative and two Gram positive indicator bacteria used for antimicrobial assay respectively, *Escherichia coli* (*E. coli*) (MTCC 443), *Klebsiella pneumoniae* (*K. pneumoniae*)(MTCC 109), *Staphylococcus aureus* (*S. aureus*) (MTCC 3160) and *Streptococcus mutans* (*S. mutans*) (MTCC 890) were provided by microbiological laboratory and clinical detection center Midnapur (West Medinipur, India). They were cultured in tryptone soy broth or agar (TSB or TSA) in aerobic condition at 37 °C.

2.3. Preparation of methanolic extract of bark of T. arjuna

The collected *T. arjuna* barks were cut into small pieces. The plant parts were dried in an incubator for 7 d at 40 °C, crushed in an electrical grinder and then the powder was separated. A total of 100 g of bark powder of said plant material was washed in 400 mL of petroleum ether for 24 h to remove the greasy pigmented non polar materials. Then the petroleum ether was discarded and residue was dissolved in 500 mL diethyl ether for 2 h in a soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper and the resulting filtrate was dried in the air. The ether solid extract was dissolved in 300 mL acetone for 1 h in a soxhlet apparatus. Then the extract was filtered through Whatman No. 1 filter paper and the resulting filtrate was dried under reduced pressure at 40 °C on a rotary evaporator. The acetone solid extract was dissolved in 200 mL methanol and was dried in the air. The methanol extract was stored in refrigerator for phytochemical screening, antioxidant and antimicrobial activity study. Percent of yield[12] was calculated as follows:

Extract yield %= $(W_1/W_2)\times100$

Where, W_1 is net weight of powder in grams after extraction and W_2 is total weight of wood powder in grams taken for extraction.

2.4. Phytochemicals analysis

Phytochemical analysis of the test sample was carried out according to standard methods[13-15].

2.4.1. Salkowski reaction test for phytosterols

To 0.5 mL chloroform extract in a test tube add 1 mL of concentrated (conc.) H_2SO_4 from the sides of the test tube. Appearance of reddish brown colour in chloroform layer indicates presence of phytosterols.