Original Article

Isolation Of Bioactive Compounds From Terminalia Arjuna Leaves And Its Applications

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Abstract

Phytochemical screening, antibacterial activity, and cytotoxic activity of Terminalia arjuna leaf soxhlet extracts employing different solvents were studied. Phenols, flavonoids, tannins, saponins, alkaloids, glycosides, phytosterols, and carbohydrates were all found in phytochemical analysis. Antimicrobial efficacy was determined utilizing the disc diffusion technique with two different bacteria. When compared to the gold standard, extracts of ethyl acetate proved to be effective against bacteria. Cytotoxicity testing using breast cancer cell lines (MCF-7) confirmed the efficacy of a crude extract whose GC-MS analysis revealed 11 bioactive components.

Keywords: Extraction, chromatography, anti-microbial activity, GCMS, MCF-7, Soxhlet.

1. INTRODUCTION

Medicinal plants are those that have curative qualities or have positive pharmacological effects on an animal's body. The use of medicinal plants, including their fresh or dried parts, whole, chopped, powdered, or advanced forms typically generated through extraction with different solvents, plays a significant role and forms the basis of most traditional systems of therapy [1]. The term "botanical medicines" or "phytomedicines" refers to the widespread usage of any plant's seeds, berries, leaves, bark, roots, or flowers for medicinal purposes [2]. The medicinal plants that are growing beside them may not have any obvious physical characteristics, but they do have some unique attributes or virtues that make them significant in medicine. It is now known that plants with natural secondary metabolite synthesis and accumulation, such as alkaloids, glycosides, tannins, volatile oils, and vitamin and mineral content, have therapeutic effects [3]. A medical plant is any plant that has a chemical that can be utilized for therapeutic purposes in one or more of its organs, or that serves as a precursor for the production of effective pharmaceuticals, according to the World Health Organization's (WHO) consultative group on medicinal plants [4].

The Terminalia species of the Combretaceae family are well-known traditional and ethnomedicinal plants [5]. They are deciduous and evergreen trees that reach heights of 6 to 30 meters [6,7]. There are more than 250 Terminalia species in the world, and at least 50 of them are consumed as food [8]. As food supplements, Terminalia fruit and bark are employed [9]; the bark of T. bellerica and T. elliptica is used to manufacture wine and palm sugar [9]. Their fruits are frequently eaten fresh and are used in preserves. A Terminalia species that is exclusive to India, Nepal, China, Bangladesh, and Vietnam is known as Terminalia eliptica. In both dry and wet deciduous woods in southern India, it can reach heights of up to 1000 meters. It has a trunk that is 1 meter in diameter and stands 30 meters tall. The 3 cm long, ovoid fruit has five wings that do not extend past the fruit's apex. The bark is fire-resistant. The wood has a gritty texture, a fairly straight grain, a dull to slightly shiny look,

and no discernible smell or flavour. The hardwood has darker streaks and a colour spectrum from pale brown to dark brownish-blackish black. The sapwood has a characteristic pattern and is a reddish-white tint

A sizable tree called Terminalia arjuna (T. arjuna), can be found all over South Asia. This tree often has new leaves that emerge in the hot season (February to April). In Bangladesh, this tree is considered to be exotic. One of the most adaptable medicinal herbs, it exhibits a broad range of biological action. The powder of T. arjuna's bark functions as a diuretic in cases of liver cirrhosis and relieves symptoms of hypertension. The bark is also anti-dysenteric, antipyretic, astringent, cardiotonic, lithotriptic, and tonic [10]. Its leaves have been demonstrated to have analgesic and anti-inflammatory effects in trials on mice [11].

The study's objectives were to determine the chemical composition of the extract and assess its biological activity when derived from the leaves of T. arjuna.

2. MATERIALS AND METHODS

Sample Preparation:

This study was carried out on T. arjuna leaves. Collection was done on April 2022 from Gokarn̄a 14°33'0.00" N 74°19'0.01" E, Karnataka. Leaves were allowed to dry in the dark shade for about 14-15 days and later crushed into small pieces.

Extraction by Soxhlet Apparatus:

Isolation of desired content when other chemicals or impurities are insoluble in the solvent is made possible by the Soxhlet apparatus. The Soxhlet apparatus, made of glass, consists of a round-bottomed flask, an extraction chamber, a syphon tube, and a condenser at the top. The plant matter is packed inside a cotton thimble, which is a porous bag, that is tightly closed. Extracting secondary metabolites with ethyl acetate. Once the bioactive components have been entirely removed, the solvent pouring out of the extraction chamber will no longer leave any trace. This process is repeated for roughly 15–20 cycles and subjected to evaporation in Rota evaporator

Thin Layer Chromatography:

A drop of sample is taken from the Eppendorf tube and it is transferred into a silica gel slide and then it is placed in the beaker of a solution containing a mixture of ethyl acetate and hexane ensuring that there will be no contact between the drop and the solution.

Phytochemical components:

With the help of established techniques, we checked several extracts for the presence of phenols, flavonoids, tannins, saponins, alkaloids, glycosides, phytosterols, and carbohydrates [12].

Preliminary Tests:

To establish the existence of bioactive secondary metabolites in the ethyl acetate extract of the plants, preliminary phytochemical profiling was conducted. According to previously described procedures, the presence of several phytochemicals was examined using a conventional biochemical assay.

Determination of Antibacterial Activity

Extracts' antibacterial activity was measured using the disc diffusion technique [13]. Bacterial strains representing both gram positivity and gram negativity were employed in the experiment. The test samples were dissolved in solvents of known volumes to create solutions of known concentration (mg/mL). After the filter paper discs had been dried and sanitized, a micropipette was used to saturate them with the test compounds in precise quantities. The discs with the test substance were placed on agar plates that had been uniformly seeded with the microorganisms. Kanamycin discs, a standard antibiotic disc, served as a positive control. The plates were placed in a 37°C incubator for 24 hours to promote optimal microbial growth. The antibacterial test materials stifled the growth of the microorganisms, as evidenced by the presence of a definite zone of inhibition all the way around the medium. The diameter of the zone of inhibition, in millimeters, was used to calculate the antibacterial activity.

Thin layer chromatography

As per standard procedure is followed and maintained the solvent ethyl acetate and hexane (1:4 ratio)

GC-MS Analysis

Phytochemical analysis and chemotaxonomic studies of medicinal plants with biologically active components rely heavily on gas chromatography-mass spectrometry (GC-MS), a combined analytical technique used to quantify and identify chemicals contained in a plant sample.

Phytochemicals have anticancer properties.

Plant extracts were evaluated for their cytotoxic effects specifically on breast cancer cell lines (MCF-7). The soxhlet extract's potential anticancer effects were examined. It has been shown that chemicals derived from plants can decrease cancer cell activity by, for example, preventing the growth of cancer cells or causing them to commit suicide (apoptosis) [14].

MTT Assay: It is now generally recognized that tetrazolium salt reduction is a valid method for analysing cell proliferation. To produce reducing equivalents like NADH and NADPH, metabolically active cells reduce the yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide). Solubilization and spectrophotometric quantification of the resultant intracellular purple formazan are possible. When metabolic events cause cell death, such as apoptosis or necrosis, the assay evaluates the rate at which cell viability decreases [15].

Synthesis of AgNPs

For the synthesis of AgNPs, about 1 ml of the extract was added to 25 ml of 0.01 M $AgNO_3$ solution at room temperature.

3. RESULTS AND DISCUSSION

Phytochemical screening revealed the presence of bioactive compounds (Table 1).

Table 1: Result of chemical group test of various extracts of leaves of T. arjuna.

Test	Result
1. Alkaloids:	+
Wagener Test	
Mayer's Test	-
Hager Test	+
Tannic Acid Test	+
Dragendorff Test	-
2. Flavonoids:	-
Shinoda Test	
Alkaline Reagent Test	+
Ammonium Test	+
Lead Acetate Test	-
3. Saponins:	-
Emulsion Test	
Frothing Test	+
4. Steroids:	+
Salkowski Test	
5. Triterpenoids:	+
Salkowski Test	
6. Tannins:	-
Ferric Chloride Test	
Gelatin Test	+
Braemer's Test	-
7. Phenols:	-
Ferric Chloride Test	
8. Glycosides:	+
Legal Test	
Kellar- Killiani Test	+
9. Anthraquinones:	-
Borntrager's Test	
KOH Test	+

10. Cumarins:	-
11. Diterpenes:	+
12. Anthocyanosides:	-
13. Resins:	-
14. Volatile Oil:	-

Antimicrobial Screening: There is evidence that some herbs used in Indian medicine have antibacterial properties [16, 17]. The result of the antimicrobial screening of ethyl acetate extracts of T. arjuna has shown in Table 2. When compared to standards the activity of plant extract was less but active to show activity (Figure 1).

Table 2: Antimicrobial activity of T. arjuna extract

Microorganisms	**Control**	**Test**
B. cereus	15mm	05mm
E. coli	10mm	05mm

(A) Gram –ve Bacteria

(B) Gram +ve Bacteria

Figure: 1 -Antibacterial activity of plant extract

UV Analysis: UV-Vis spectroscopy is used to detect chromophores and aromatic rings (Figure 2). The presence of UV-Vis absorption bands indicative of alkaloids, flavonoids, phenolic acids, and tannins in plant extracts was discovered [14, 18].

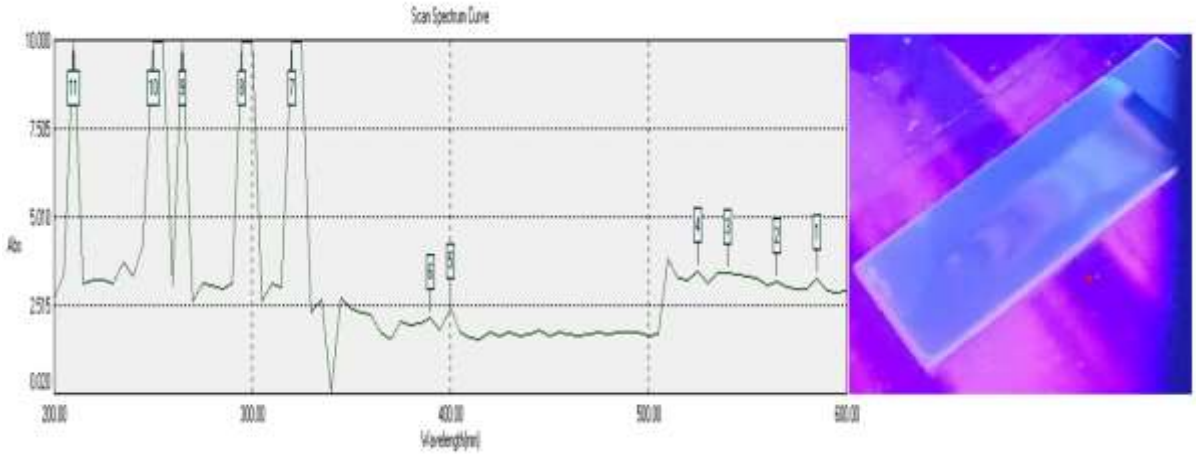

Figure: 2 - UV-Vis spectroscopy

Absorption spectra showed many bands at 265 nm for the pure reference chemical gallic acid, 250 nm and 370 nm for quercetin and rutin, and 275 nm for tannic acid. Both the 270-280 nm and 350-500 nm absorption bands are indicative of tannins and flavonoids, whereas the 400-450 nm absorption band is thought to be due to carotenoids [18]. Consequently, UV-Vis spectroscopy was used to successfully identify the chromophoric groups present in molecules isolated from a wide range of plant sources. With the help of thin layer chromatography, we can say there probably around 11 compounds are present.

GC Analysis

Table 3 displays the results of GC-MS analysis of the ethyl acetate plant leaves extract, including a chromatogram showing the peaks of the various chemicals found in the GC fractions (Figure 3). There were 11 distinct bioactive chemicals found in this sample, including, Dodecane, 4,6-Dimethyl- Nonadecane, Phenol, 3,5-Bis(1,1-Dimethylethyl)-2,6,10,15-Tetramethylheptadecane, Tridecanol, 2-Ethyl-2-Methyl-Docosane, 1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester, 1,2-Benzenedicarboxylic Acid, Dibutyl Ester, 2-Bromotetradecane, Pentadecane, Dotriacontane. The evidence shows that these compounds are medicinally important

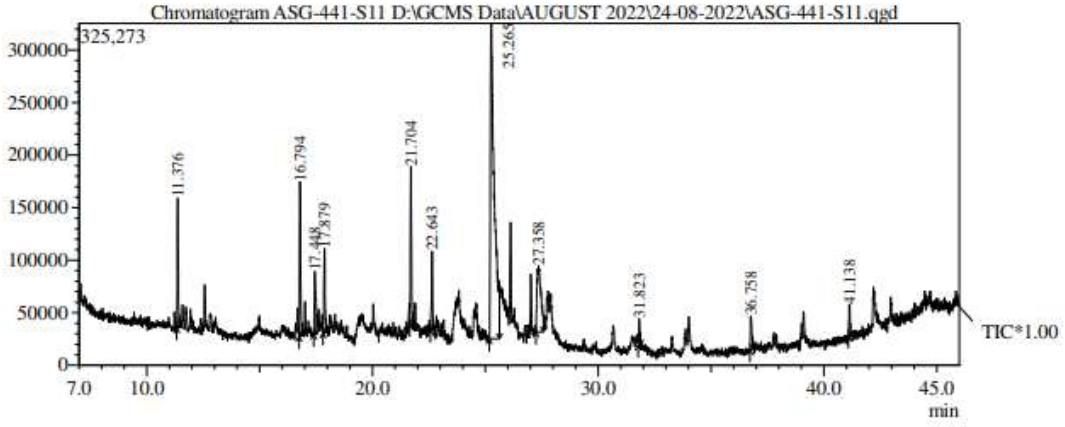

Figure : 3 -GC-MS chromatogram

Table 3: GC-MS analysis of the ethyl acetate plant leaves extract

Peak#	R. Time	Area	Area %	Height	Height%	Name	Base m/z
1	11.376	338159	5.04	123250	11.35	Dodecane, 4,6-Dimethyl-	57.10
2	16.794	453384	6.75	147348	13.57	Nonadecane	57.10
3	17.448	280350	4.18	59659	5.49	Phenol, 3,5-Bis(1,1-	191.10

						Dimethylethyl)-	
4	17.879	284539	4.24	80241	7.39	2,6,10,15-Tetramethylheptadecane	57.10
5	21.704	428103	6.38	150262	13.84	Tridecanol, 2-Ethyl-2-Methyl-	57.10
6	22.643	244100	3.64	74974	6.90	Docosane	57.10
7	25.265	3609959	53.78	299873	27.62	1,2-Benzenedicarboxylic Acid, Bis(2-Methylpropyl) Ester	149.00
8	27.358	749581	11.17	62980	5.80	1,2-Benzenedicarboxylic Acid, Dibutyl Ester	149.00
9	31.823	83702	1.25	25161	2.32	2-Bromotetradecane	57.10
10	36.758	147112	2.19	32027	2.95	Pentadecane	57.10
11	41.138	93347	1.39	30026	2.77	Dotriacontane	57.10
		6712336	100.00	1085801	100.00		

Anticancer activity: Plant extracts' cytotoxic potential was evaluated in MCF-7 breast cancer cell lines. There was an examination of the crude extract for its potential anticancer effects [15]. When compared to conventional cancer treatments, plant-derived compounds have been shown to inhibit cancer cell activity by, for example, preventing the proliferation of cancer cells and inducing apoptotic cell death shown hown in Figure 4

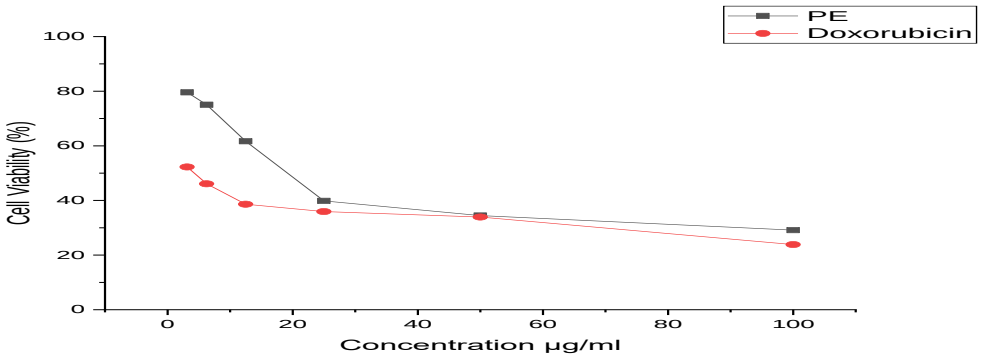

Figure:4 – MTT assay

Silver nanoparticle synthesis

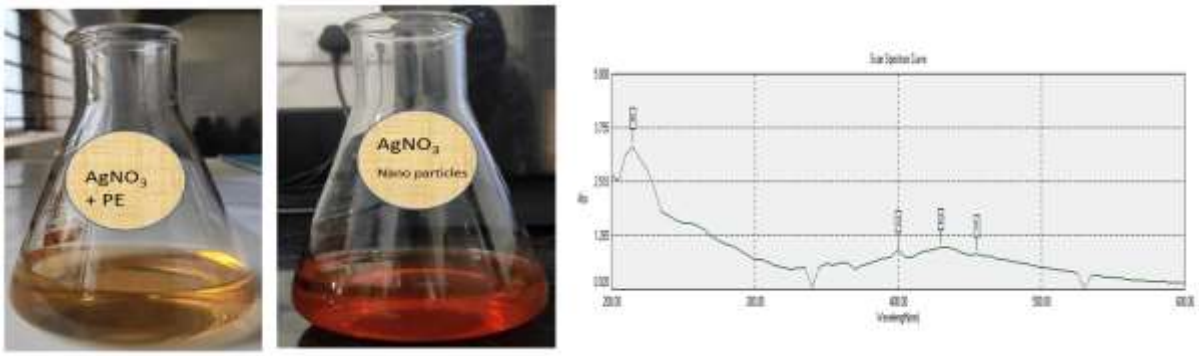

Figure 5 : UV-Vis spectrum of nanoparticles synthesized using T. arjuna leaf extract.

When exposed to plant extract, silver ions are converted into silver nanoparticles, which causes the color change. The color variation is brought on by the surface Plasmon Resonance phenomena. The free electrons in the metal nanoparticles that produce the SPR absorption band are due to their synchronous vibrating in resonance with the light wave[18-20]. In the case of the leaves of T. arjuna, the sharp band of silver nanoparticles was found to be around 430nm (Figure 5).

4. CONCLUSION

The results of the current investigation suggest that T. arjuna extracts have cytotoxic and antibacterial capabilities. According to GCMS analysis, there may be 11 molecules that are bioactive among the chemical constituents in

ethyl extract. In high quantities, T. arjuna extracts are effective against both gram-positive and gram-negative bacteria. T. arjuna contains bioactive components, as shown by the MTT experiment.

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No conflict of interest.
Contribution of authors
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