

Analysis of Phytoconstituents Present in Terminalia arjuna Bark Extract Using Spectroscopic Techniques

B. Anita^{1*} and V. Renganayaki²

¹Department of Physics, Queen Mary's College, Chennai – 600 004, India

²Department of Physics, D. G. Vaishnav College, Chennai- 600 106, India

Abstract : Plants have been in use as medicines since the beginning of human culture. Plant extracts have been used as medicines to treat a wide range of ailments, ranging from cancer to pain management. One of the most widely used plant to treat heart ailment is *Terminalia arjuna*. The *arjuna* was introduced into the Ayurveda system of medicine to treat heart disease as early as 7th century. The bark extract of the *arjuna* tree is a commonly used medicine for cardiac problems. In the present investigation, the ethanolic bark extract of the *arjuna* was subjected to spectroscopic studies such as FTIR and GC-MS and the structure of various phytochemicals present in the extract were analysed. Even though the use of bark of *arjuna* plant as a cardiac medicine has been well established, its use as medicine of other ailments is still under investigation. So, as a next step in the current investigation, the anti-fungal properties of the bark extract of the *arjuna* plant has been studied by disc-diffusion method and the results have been discussed.

Introduction

Our country is endowed with a rich variety of flora that is used as medicines. Due to this reason our country is ranked as one of the top producers of herbal medicines in the world. It is documented by the World Health Organization(WHO) that above 80% of the world's population has faith in traditional medicinal forms, particularly plant - based drugs for their health care(1). In India, there are around 8000 plant species that have been recognized for their medicinal value and about 7000 firms manufacturing traditional medicines(2).

Herbal drug development includes various steps like, starting from data on raw materials, correct identification of plant species, checking chemical quality, standardization and clinical trials. There are two major issues of concern regarding the development of herbal medicines. They are

- Medical plants are generally collected following traditional methods without adhering to the pharmacopoeial standards laid down for the collection of different parts of a plant. So there is no control on the quality of raw material collected. According to standard practice, tubers, corns, and bulbs are to be collected at the end of the flowering season. Tree bark/branches must be collected after the proper maturity stage of the tree. But, collection of plant parts for preparation of medicines is mostly done by farmers whose knowledge about such standard practice is very limited (3).

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- The next issue is adulteration or substitution of the crude herbal drugs. Adulteration in market samples and availability of sub-standard products is one of the greatest drawbacks in promotion of herbal products from India. So, checking for adulteration and standardisation of manufactured drugs is very vital. Most of the plants used in our indigenous system of medicine are being used since ancient times and are mentioned in Ayurvedic literature but, there is a lack of proper description and voucher specimens for most of these plants in this literature. There are many examples of botanically different plants linked with one Indian name, eg: The name *Ashok-Polyalthia longifolia* and *Saraca indica*. Also *terminalia arjuna* and *terminalia alata* are both marketed as *arjun* an ayurvedic medicine used for the treatment of cardiac ailments.

In view of the above issues, in the present study, an attempt has been made to show that spectroscopic techniques like FT-IR, GC-MS can be used for quality analysis of herbal medicines. The *Terminalia arjuna* tree whose bark is a popular Ayurvedic medicine for heart ailments has been chosen for spectroscopic analysis. The FTIR and GC-MS spectra of the samples have been analysed and various functional groups have been identified by comparison with standard data. The spectra can be used as standard for comparison with commercial samples available in market to identify their authenticity and purity. *Terminalia arjuna* is an evergreen tree found all over our country in deciduous forests (4). The bark of the tree has the capacity to even reverse heart failure. It is called guardian of heart. As it is the most effective medicine in Ayurveda to treat cardiac problems as already mentioned, *Terminalia arjuna* is replaced by another species of the same genus, *Terminalia alata* marketed as Ayurvedic medicine used for the treatment of cardiac ailments. Therefore, it becomes necessary to test market samples of the medicine for purity. Earlier researchers have reported spectral analysis and chromatographic studies on the active compounds present in the bark (5-8), but, so far no work on the GC-MS analysis of bark extract of the tree has been reported.

Experimental

In order to perform spectroscopic analysis of bioactive phytochemicals present in the tree, the bark of the tree was collected under the guidance of a botanist and the alcoholic extract of the bark was taken. After taking alcohol extract of the bark sample, it was subjected to spectroscopic analysis (FTIR, GC-MS). The presence of bioactive compounds can be confirmed by analyzing its GC-MS and FTIR spectra.

The use of bark of *Terminalia arjuna* as cardiac medicine has been proven by many researchers beyond doubt (9, 10). But, the use of the plant to cure other common ailments is yet to be explored. With the intention of testing the use of the plant as a potential anti-microbial medicine, the anti-fungal activity of the plant has been tested in this work. The anti-fungal activity of the bark extract prepared in ethanol was determined against common disease causing fungi, *Candida albicans* and *Aspergillus niger*.

About *Terminalia arjuna*:

Terminalia arjuna is a tree of the genus *Terminalia*. It is commonly known as *arjuna* or *Arjun* tree in English. *Terminalia arjuna* tree bark is used medically in Ayurveda for the purpose of cardiovascular health. It has a large variety of bioactive components. *Arjun* is a large sized deciduous evergreen tree with very strong and long roots. This tree reaches height upto 70.85 feet. It has conical leaves, yellow flowers and grey colored smooth bark. It has glabrous, fibrous woody, 2-3cm long fruits with five hard wings and number of curved veins. Leaves are dull green above and pale brown beneath. Flowers of *arjuna* appear between September to November.

Therapeutic Effects:

There are numerous human studies conducted on *arjuna* bark. Nevertheless, the water extract appears to be effective in improving cardiac function in persons who have recently undergone cardiac trauma. Myocardial infarction is the most commonly researched ailment in this regard. *Arjuna* showed benefits in improving left ventricle function in an exercise test and the benefits may affect a person regardless of health state. The bark is cardio protective, cardio tonic, removes obstruction from fluid channel, astringent and capable of reducing fever. It is effective in treatment of elevated blood pressure, palpitation of heart and angina (11 – 12). *Arjuna* bark tones the heart, stimulates blood circulation, stops bleeding and strengthens the heart muscle. Other extracts such as ethanolic or acetone, with different bioactive components appear to be somewhat cancer protective.

In view of such wide medicinal uses of the tree, the present work was undertaken, to carry out a thorough spectroscopic analysis of the different phytochemicals present in the plant, that are responsible for its therapeutic effects.

Preparation of Plant extract:

Extraction is the separation of medicinally active portion of plant using selective solvent through standard procedure.

The *Terminalia arjuna* plant was identified by a botanist, and the bark of the plant was collected. It was shade - dried at room temperature. Then, the dried bark was crushed separately in electrical grinder and then the powder was separated. A total of 5g of bark powder was dissolved in 50ml of ethanol and stirred in magnetic stirrer about 10 hours, to get a solution of uniform concentration. Then the solution was filtered twice to obtain the required bark extract of *Terminalia arjuna* plant. This extract was used for spectroscopic studies.

FTIR Analysis of alcoholic extract of *arjuna* bark

The vibrational spectrum of a compound is the superposition of the absorption bands of specific functional groups. Raman and infrared spectroscopy are complementary techniques, which provide a complete impression of the biochemical information within a sample (12). The functional groups present in the bark extract of *Terminalia arjuna* tree were identified and a satisfactory vibrational band assignment of the sample has been made by comparing spectra with those that are already available and also by observing the nature, shape and intensity of the vibrational bands in the spectra (13, 14).

The FTIR spectra of the bark extract of *Terminalia arjuna* was recorded in the range 4000-400 cm^{-1} at Queen Mary's college, Chennai.

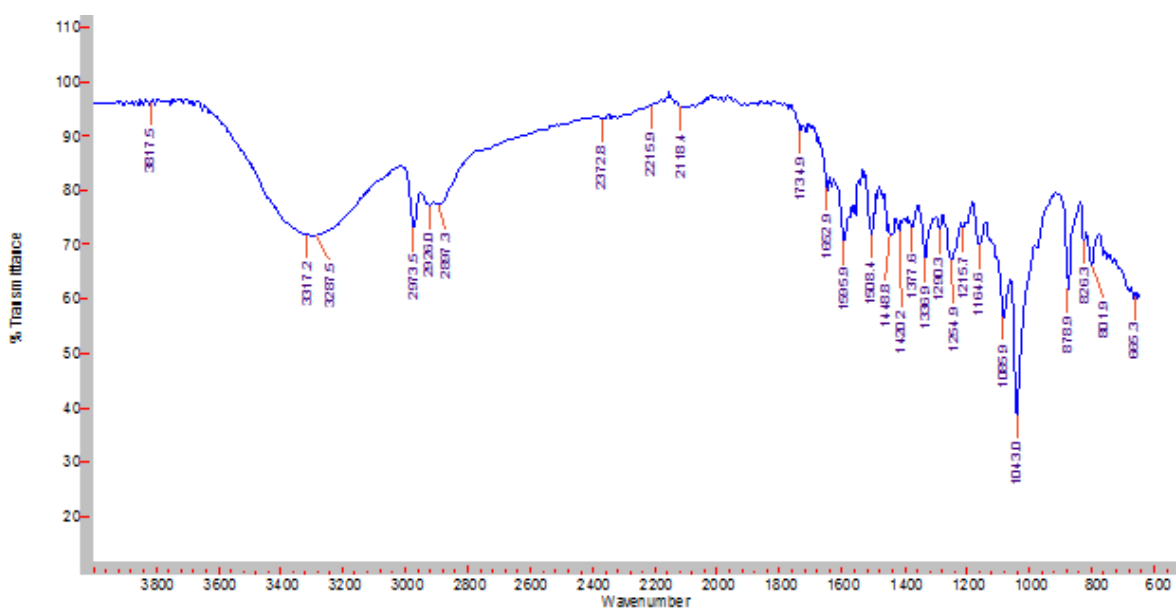


Fig.1. FTIR Spectrum of bark extract of *Terminalia arjuna*

The spectrum of ethanolic extract of bark of *Terminalia arjuna* is given in Fig. (1). The spectrum revealed the existence of various functional groups of different phytoconstituents contained in the bark extract. The vibrational frequencies have been assigned to different functional groups. The vibrational band assignment is given table 1.

Table 1. Vibrational band assignment for bark extract of *Terminalia arjuna*

Bark extract	Vibrational band assignment
3317	O-H stretching
3287	O-H stretching
2973	Aromatic C-H stretching
1734	C=O stretching
1652	N-H deformation
1595	C=C stretching
1508	NO ₂ stretching (asymmetric)
1448	NO ₂ stretching (asymmetric)
1337	NO ₂ stretching (symmetric)
1336	C-N stretching of primary amine
1254	C-H deformation (in plane)
1215	C-H deformation (in plane)
1164	C-H deformation (in plane)
1085	C-F stretching (very strong)
1043	C=S stretching
878	C-H deformation (out of plane)
826	C-H deformation (out of plane)
801	C-H deformation (out of plane)

C=O group (carbonyl group):

All C=O absorptions are strong. The group occurs in many classes of organic compounds, and gives rise to strong absorption in a region where few other bands are found. The overall spread of carbonyl group frequencies is wide, from 1928 cm⁻¹ to 1580 cm⁻¹. Aldehydes may be differentiated from ketones by the presence of C-H stretching absorption of the CHO group at about 2800-2700 cm⁻¹, carboxylic acids are usually identified by means of the broad, O-H stretching absorption at 3000 cm⁻¹ and the coupled C-O stretching O-H deformation bands at 1420 cm⁻¹ and 1300-1200 cm⁻¹ (15).

Amine group:

The N-H bond like O-H participates in hydrogen bonding, although the electro negativity of nitrogen weakens the effect. The -NH₂ group in dilute solution in a non-polar solvent gives rise to two bands –anti symmetric and symmetric free N-H stretching. So we can distinguish it from a secondary amine, which is transparent in the region. In the present work, broad bands at 3817 cm⁻¹ has been assigned to -NH₂ stretching (16).

Vibrational band assignments of other frequencies were made based on similar work by previous researchers.

GC-MS Analysis:

The analysis of complex mixtures of plant extracts are usually performed by Gas Chromatography (GC) or the combined technique of Gas Chromatograph-Mass Spectrometry (GC-MS), which utilizes the separating power of GC with MS to yield the molecular ions of components of a mixture.

GC-MS analysis of *T.arjuna* bark extract predicted the presence of 12 phyto- chemicals, at different retention time. At a particular retention time, components were separated out according to their mass/charge ratio. The mass spectra of the components were matched with the data available in the National Institute of Standards and Technology (NIST) library. Results are tabulated in Table 2. Most of the phytochemicals of therapeutic importance fall under the broad category of terpenes, flavonoids, glycosides and phenols. Some of the common class of phytochemicals found in the GC-MS spectrum of *T. arjuna* bark extract are discussed below:

Table. 2. Compounds present in the bark extract of *Terminalia arjuna*:

Peak	R. Time	Chemical Formula	Compound	Structure
1	14.57	C ₁₂ H ₂₄ O	7-Dodecen-6-one	
2	16	C ₁₂ H ₂₂ O	Phenol, 2,4-bis(1,1-dimethylethyl)	
3	16.4	C ₁₅ H ₁₆ O ₂	Flavone	
4	16.8	C ₁₆ H ₃₂	1-Pentadecene,2-methyl	
5	17.55	C ₁₈ H ₂₃ O	Estra-1,3,5(10)-trien-17α-ol	
6	18.63	C ₁₉ H ₃₆ O	Z,E-2-Methyl-3,133-octadecadien-1-ol.	
7	19.53	C ₁₆ H ₁₂ O ₅	Acacetin	
8	20.02	C ₁₉ H ₃₆ O ₃	Octadecanoic acid, 3-oxo,methyl ester	
9	21.13	C ₂₁ H ₄₄ SiO	Octadec-9Z-enol trimethylsilyl ether	
10	22.52	C ₂₀ H ₁₈ O ₆	3,4-Dihydroxy-1,6-bis-(3-methoxy-phenyl)-hexa-2,4-diene-1,6-dione	
11	24.33	C ₂₁ H ₄₀ O ₄	1,13,Dioxacyclotetracosane -2,14-dione	
12	27.7	C ₂₄ H ₄₆ O ₂	Cyclopropanedodecanoic acid, 2-octyl,methyl ester	

Terpenes:

The terpenes are widespread in nature and occur in most natural species. Terpenes contribute to many of the aromas associated with plants and range in complexity from simple C₅ units (isopentane) to polyisoprenes which include latex, leaf waxes and rubber. The terpenes are perfect example of a natural product class that is highly structurally diverse , has many members that are chiral and have extensive functional group chemistry.

Glycosides:

The term glycoside is a generic term for a natural product that is chemically bond to a sugar. It is thus composed of two parts : the sugar and the aglycone. The aglycone may be a terpene, a flavonoid or any other natural product.

Alkaloids are a group of natural products that find wide use in medicines and pharmaceutical preparations. They display an exceptionally wide array of biological activities and have an equally wide

distribution. They are present in plants, fungi, bacteria and even marine animals. Alkaloids may also be defined as heterocyclic natural products containing nitrogen, including compounds that contain nitrogen in the aliphatic chain.

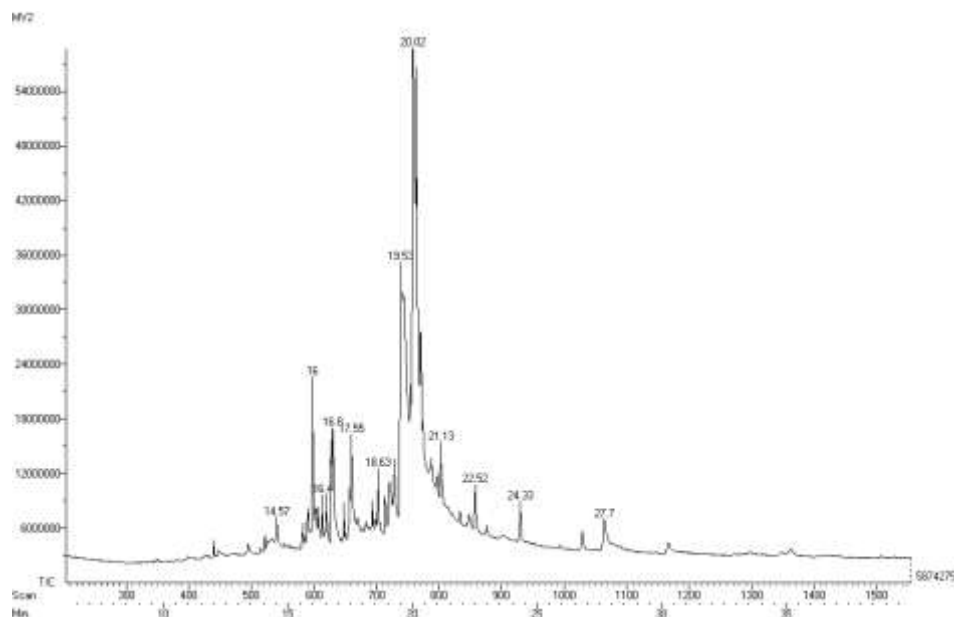


Fig. 3. GC-MS Spectrum of bark extract of *Terminalia arjuna*

Anti-fungal activity studies of *Terminalia arjuna* bark extract:

Many plant extract of different species have been studied and are found to exhibit antimicrobial properties. India, being a tropical country with a large population, infections and diseases caused by microorganisms are a cause of major concern affecting the living standard of the population. Hence a lot of research is being carried out develop effective anti-microbial medicines from plant extracts. A number of different solvent systems like water, ethanol, chloroform, methanol, petroleum ether have been reported to play important role for extraction of secondary metabolites, such as tannins, terpenoids, alkaloids, flavonoids, phenols and quinones. *Treminalia arjuna*, traditionally has been used as a cardio tonic. Bark of *T. Arjunahas* been broadly used in traditional system of medicine for various purposes that have been already discussed.

In present study, an attempt has been made to investigate the antimicrobial activity of *Terminalia arjuna* extract against two microorganisms, fungi *Candida albicans* and *Aspergillus niger*. These two fungal species were chosen because they are the common infections that affect plants and animal species in tropical countries like ours (17, 18).

Agar disc diffusion method:

This method is based on the principle that antibiotic-impregnated disk, placed on agar previously inoculated with the test bacterium, pick-up moisture and the antibiotic diffuse radially outward through the agar medium producing an antibiotic concentration gradient. The concentration of the antibiotic at the edge of the disk is high and gradually diminishes as the distance from the disk increases to a point where it is no longer inhibitory for the organism, which then grows freely. A clear zone or ring is formed around an antibiotic disk after incubation if the agent inhibits bacterial growth.

Antifungal activity of the Sample was determined by disc diffusion method on Sabouraud Dextrose Agar (SDA) medium (19, 20). Sabouraud Dextrose agar (SDA) medium is poured into the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. Amphotericin-B is taken as positive control. Samples and positive control of 20 μ L each were added in sterile discs and placed in SDA plates. The plates were incubated at 28°C for 24 hrs. Then antifungal activity was determined by measuring the diameter of zone of inhibition. The experiment was carried out at Life Tech Research Centre, Arumbakkam, Chennai.

Determination of Antifungal activity of Plant extract:

In present study the antimicrobial activity of *T.Arjuna* bark extracts prepared in ethanol was determined against fungus *Candida albicans* and *Aspergillus niger*. Fig. 4 shows the zone of inhibition of the bark extract against the fungi mentioned above. The results presented in Table. 4 revealed that both extracts display potential antifungal activity against tested microorganisms.

Table 3. Antifungal activity for bark extract of *Terminalia arjuna*

Organism	Zone of inhibition (mm)			Antibiotic (1mg/ml)
	Concentration (µg/ml)			
	1000	750	500	
<i>Aspergillus niger</i>	10	-	6	22
<i>Candida albicans</i>	9	6	7	12



(a) for *Aspergillus niger*

(b) *Candida albicans*

Figure. 4 Zone of inhibition of bark extract for (a) *Aspergillus niger* and (b) *Candida albicans*

Conclusion:

The present investigation presents a complete spectroscopic analysis of ethanolic extract of bark of *Terminalia arjuna*, a proven heart tonic in the Ayurveda system of medicine. The FTIR and GC-MS spectra reveal the presence of many phytoconstituents belonging to terpenes, glycosides and other important functional groups. Thus, the spectroscopic studies have given useful information about the phytochemicals present in the sample. As a next step, the anti-fungal activity of the bark extract has been investigated by using the disc-diffusion method. The efficacy of the bark extract against common fungi present in tropical countries, like *Aspergillus niger* and *Candida albicans* has been investigated, and the efficacy of the bark extract as an anti-fungal agent is proven.

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