Phytochemical Analysis and Anti-microbial Studies of Leaf Extract of *Terminalia arjuna* using Spectroscopic Methods

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Abstract : From time immemorial plants have been used as potent therapeutic agents to treat various ailments in the Siddha and Ayurveda streams of medicine. Plants are rich sources of flavonoids, alkaloids, terpenoids and other related polyphenols. One such important plant used for treating heart ailments is *Terminalia arjuna.* The bark of this plant plays a vital role as cardiac tonic. It also possesses antimicrobial, cytotoxic and antidiabetic properties. The phytochemical activity of the secondary metabolites present in the bark of *T.arjuna* has been reported widely. But, the medical uses of leaf extract of the plant have seldom been reported. In this work, the ethanolic extract of leaf of *T. arjuna* has been analysed for the presence of different phytochemicals using FTIR, UV-Visible and GC-MS spectroscopic methods. The various functional groups present in the leaf extract have been identified initially using FTIR and UV-Vis spectra of leaf extract. Next, GC-MS studies revealed the complete structure of the twelve major phytoconstituents that were present in the leaf extract. In the next stage of the study, the therapeutic effect of the extract as potent anti-bacterial and anti-fungal agent has been studied. The presence of a flavonoid and ester groups in the leaf extract as revealed by the GC-MS analysis and the potential anti-fungal and anti-baterial activity of the extract shows that leaf extract of *Terminalia arjuna* has a great potential to be used as a therapeutic agent.
Keywords : Anti-microbial activity – FTIR – GC-MS – phytoconstituents - *Terminalia arjuna* - UV-Visible spectrum.

Introduction:

Plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as medicinal plants. In most of the traditional systems of treatment, the use of medicinal plant include the fresh or dried part of seeds, berries, leaves, bark, root or flowers of any plant, whole, chopped, powdered and extracted with different solvents like hot water, ethanol. Such plant extracts play a major role in treating many diseases that affect animals and human beings .These constitute the backbone of traditional medicine. It has now been established through many years of research that the plants which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins and volatiles oils possess medicinal properties (1). According to The World Health Organization (WHO) "A medicinal plant is any plant which in one or more of its organs contains substance that can be used for therapeutic purpose or

which is a precursor for synthesis of useful drugs"(2). Medicinal plants constitute an important natural wealth of a country. They play a significant role in providing primary health care services to rural people. They serve as therapeutic agents as well as important raw materials for the manufacture of traditional and modern medicine. Substantial amount of foreign exchange can be earned by exporting medicinal plants to other countries. In this way indigenous medicinal plants play significant role of an economy of a country.

India always stands as a golden mark of well-recorded and well-practiced knowledge of traditional herbal medicine. Herbal plants play an important role in the development of potent therapeutic agents. Over the years, various medicinal plants and their extracts have been reported to be effective in the treatment of diseases. Plants are rich sources of antioxidants such as flavonoids, alkaloids, terpenoids and other related polyphenols. These phytoconstituents have potential to treat number of diseases and seem to possess no known side-effects unlike chemical – based allopathic medicines. Many plants like neem, aloe vera, tulsi, centella, turmeric are commonly used home-remedies for many common diseases since ancient times. One such important plant, well known for its therapeutic property is the *Terminalia arjuna*, commonly known as *arjuna.* It possesses antimicrobial, cytotoxic, antidiabetic, antidiarrheal, antidysentric and hepatoprotective activities (3).

Ethnopharmacognosy: *Terminalia arjuna* is a large evergreen tree about 60 – 80 feet high found throughout the greater part of Indian peninsula along river beds. Region-wise, it is found in Chota Nagpur, Orissa, West Bengal, Punjab, Deccan and Konkan region (4).

The bark appears grey and smooth on external surface. The leaves are sub-opposite and oblong or elliptic in shape. The flowers are white/yellowish and found in groups. The flowering season of the plant is summer.

Fig. 1 Leaf of *Terminalia arjuna*

Bioprospectus: *Terminalia arjuna* is known to possess cardioprotective and cardio-strengthening properties since Vedic period. It is widely used throughout India in plant-based indegenious systems of medicine like Ayurveda and Siddha. It is used for prevention of myocardial infarction. It has anti-atherogenic property, which aids in reducing plaque build-up in the coronary arteries and improves blood flow to heart tissues. In Ayurvedic branch of medical practice, *arjuna* extract is recommended for people suffering from diabetes mellitus and hypertension, as a preventive medicine for heart disease. It aids in preventing heart disease by lowering serum concentration of total cholesterol, LDL cholesterol and triglycerides (5-8). The bark extract of the plant is also used to treat anemia, tumours and hypertension. Singh *et al* (9) have reported the cardio-protective activity of butanolic fraction of *Terminalia arjuna* bark.

Secondary metabolites, commonly called bioactive phytochemicals are the reason for the therapeutic effect of plants. Therefore identifying them becomes imperative from the point of view of extracting them for manufacture of medicine. In recent years, spectroscopic techniques like FTIR, UV-Vis and GC-MS are used for the purpose of identification of phytoconstituents of medicinal plants. Since mass production of herbal drugs is required to cater to increasing number of people using alternate medicines nowadays, easy, quick, economic and accurate analytic techniques are required for identification, authentication and standardisation of active ingredients in plants. FTIR and GC-MS are the tools currently used which suit the above requirements. FTIR spectrum is generally called the 'fingerprint' of the compound analysed. The spectrum helps us to identify the functional groups of the active compounds present in the plant extract, based on the wavelength region where the peaks are present. In Mass Spectrometer, compounds are eluted at different times based on their m/z ratio.

There have been numerous reports on the therapeutic effects of the bark extract of the pIant, but so far, not much research has been reported on the analysis of the leaf extract. Therefore in the current study, a complete spectroscopic analysis of ethanolic extract of *Terminalia arjuna* leaf extract has been conducted using FTIR, UV-Vis and GC-MS techniques and the bioactive constituents have been identified. Also, the anti-bacterial and anti-fungal activity of the leaf extract has been examined by the disc-diffusion method.

Experimental:

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

FTIR Spectral Recording: FTIR analysis of the leaf extract was done for identifying the functional groups present in the sample. The FTIR spectrum of the leaf extract was recorded in the 4000 cm^{-1} to 450 cm^{-1} region at SAIF, IIT Madras, using Perkin – Elmer Spectrometer by KBr pellet method.

GC-MS Spectral Recording: The GC-MS spectrum of the sample was recorded at SAIF in IIT Madras using JEOL GC Mate II instrument and the resulting structures were compared with data available in NIST library and the compounds have been identified and named using this data.

UV-Vis Spectral Recording: The UV-Vis spectrum of the leaf extract was recorded in the wavelength range 200 – 800nm at room temperature using Perkin Elmer spectrophotometer at FIST laboratory in Queen Mary's college, Chennai.

Results and Discussion:

I. FTIR analysis: All compounds, organic and inorganic have a tendency to absorb radiation of different frequencies from the electromagnetic spectrum. Radiations from the infrared region (400 – 4000 cm^{-1}) can be utilised for structure determination by making use of the fact that these are absorbed by interatomic bonds in organic compounds. Chemical bonds in different environments will absorb radiation of different frequencies and with varying intensities. IR spectrum of a compound is information about collective absorption of IR radiation by the compound, in the form of a spectrum. Information about different functional groups present in the compound and the possible interatomic bonds can be interpreted by comparing the spectrum with data already available. There are also certain selection rules which are used to assign various stretching and bending vibrations of a molecule to different IR absorptions. The spectrum of the leaf extract of *Terminalia arjuna* is projected in Fig.1 and the vibrational band analysis is shown in Table 1.

Aromatic Compounds: Aromatic compounds show useful characteristic infrared bands in five regions of the mid-infrared spectrum. The C–H stretching bands of aromatic compounds appear in the 3100–3000 cm^{-1} range, making them easy to differentiate from those produced by aliphatic C–H groups which appear below 3000 cm^{-1} (10). In the 2000–1700 cm^{-1} region, a series of weak combination and overtone bands appear and the pattern of the overtone bands reflects the substitution pattern of the benzene ring. Skeletal vibrations, representing C=C stretching, absorb in the 1650–1430 cm^{-1} range (11). The C–H bending bands appear in the regions 1275–1000

cm^{-1} (in-plane bending) and 900–690 cm^{-1} (out-of plane bending). The bands of the out-of-plane bending vibrations of aromatic compounds are strong and characteristic of the number of hydrogen in the ring. In the extract under study, the C-H stretching vibration appear at 2983 cm^{-1} and 2148 cm^{-1} and the CH bending vibration assigned to frequency 1242 cm^{-1} . Such CH vibrations have been assigned to lipid molecules by previous authors (12, 13)

Aldehydes and Ketones: Aliphatic and aromatic ketones show carbonyl bands at 1730–1700 cm^{-1} and 1700–1680 cm^{-1}, respectively, while aliphatic and aromatic aldehydes produce carbonyl bands in the 1740–1720 cm^{-1} and 1720–1680 cm^{-1} range, respectively (14). The position of the C=O stretching wavenumber within these ranges is dependent on hydrogen bonding and conjugation within the molecule. Conjugation with a C=C band results in delocalization of the C=O group, hence causing the absorption to shift to a lower wavenumber. Aldehydes also show a characteristic C-H stretching band in the 2900–2700 cm^{-1} range. Such bands appear at 2983 cm^{-1} in the leaf extract being analysed.

Amides: Primary amides (–CO–NH–) display two strong NH$_2$ stretching bands, i.e. asymmetric stretching at 3360–3340 cm^{-1} and symmetric stretching at 3190–3170 cm^{-1}. They also exhibit C=O stretching at 1680–1660 cm^{-1}, which is usually referred to as the amide I band. The NH$_2$ bending occurring in the 1650–1620 cm^{-1} region is called as the amide II band. Secondary amides show an N–H stretching band at 3300–3250 cm^{-1}, while the carbonyl stretching (amide I band) is observed at 1680–1640 cm^{-1}. The amide II band for secondary amides is due to the coupling of N–H bending and C–N stretching and appears at 1560–1530 cm^{-1}. A weak band which is an overtone of the amide II band appears at 3100–3060 cm^{-1}. A broad N–H wagging band also appears at 750–650 cm^{-1} [15].
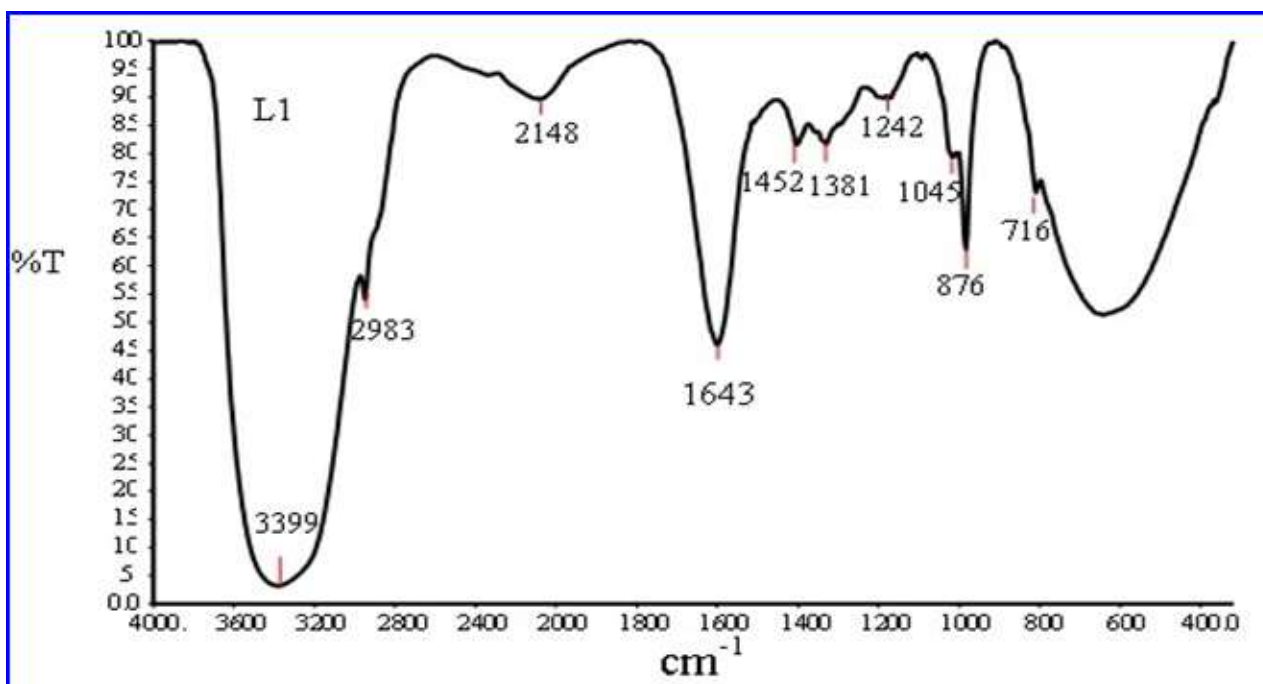

Fig.2 FT-IR spectrum of leaf extract of *Terminalia arjuna*

Table 1. Vibrational Band Assignment for leaf extract of *Terminalia arjuna*

WAVE NUMBER(cm^{-1})	VIBRATIONAL BAND ASSIGNMENT
3399	O-H stretching
2983	C-H stretching
2148	C-H stretching
1643	C=C stretching
1452	C=C stretching
1381	NO$_2$ stretching (symmetric)
1242	C-H bending
1045	C=S stretching
876	C-H out of plane deformation
716	C-H bending(out of plane)

II. GC-MS analysis: Alkaloids, saponins, flavonoids, tannins are some of the commonly present secondary metabolites of plants. The identification and quantification of such plant compounds has become easier and economically viable with advent of advanced techniques like FTIR and GC-MS. Recently, the analysis of complex mixtures of plant extracts are performed by 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 (16, 17).

GC-MS analysis of *T.arjuna* leaf extract predicted the presence of 12 phyto- chemicals, at different retention times. 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. The GC-MS spectrum of the sample is shown in Fig.3. Results are tabulated in Table 2. From the GC-MS spectrum, it was seen that Estra – 1,3,5(10)-trien-17a-01 ($C_{10}H_{36}O$, R_t = 17.5) and octadec-9-enoc acid ($C_{18}H_{34}O_2$, R_t = 19.03) are the most abundant. 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* leaf extract are discussed below:

Glycosides: Glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. The sugar group is known as a glycone, the non-sugar group as aglycone of the glycoside. The glycine consists of a single sugar group or several sugar groups 18). Many plants store chemicals in the form of inactive glycosides. On enzyme hydrolysis, they are activated, the sugar part is broken off and the chemical is made available for use. Many such plant glycosides are used as medications. Their therapeutic activity depends upon the chemical nature of aglycone and number of sugars.

Terpenes: Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers, and by some insects. They often have a strong odour and may protect the plants that produce them by deterring herbivores and by attracting predators and parasites of herbivores. Terpenoids (or isoprenoids) are modified terpenes as they contain additional functional groups, usually oxygen-containing. All terpenes are derived from the union of five carbon elements that have the branched carbon skeleton of isopentane. The basic structural elements of terpenes are sometimes called isoprene units because terpenes can decompose at high temperatures to give isoprene. In the current study, the peaks at 10, 11 and 12 represent compounds that have the general structure of terpenes.

Flavonoids: They are one of the largest and most widespread groups of plant secondary metabolites, with significant antioxidant properties. The flavonoids are a class of over 9000 molecules based upon a 15-carbon skeleton. Many flavonoids possess antibacterial, antifungal and antiviral activities both against both plant and human pathogens. In the GC-MS spectrum of leaf extract of *Terminalia arjuna* a flavonoid, flavone with molecular formula $C_{15}H_{16}O_2$ (R_t = 16.8) has been identified. It has been reported that 9, 12 octadecadienonic acid, a methyl ester possesses strong anti-cancer activity. A similar methyl ester 10-octadecanoic acid (R_t =

18.33) has been identified in the GC-MS spectrum of leaf extract of *Terminalia arjuna*, thereby the use of the leaf extract of the plant as a anti-cancer agent needs to be investigated further.

The presence of various bioactive compounds proves that the plant leaf can be used for isolation of individual phytochemical constituents and their pharmacological activity can be evaluated.
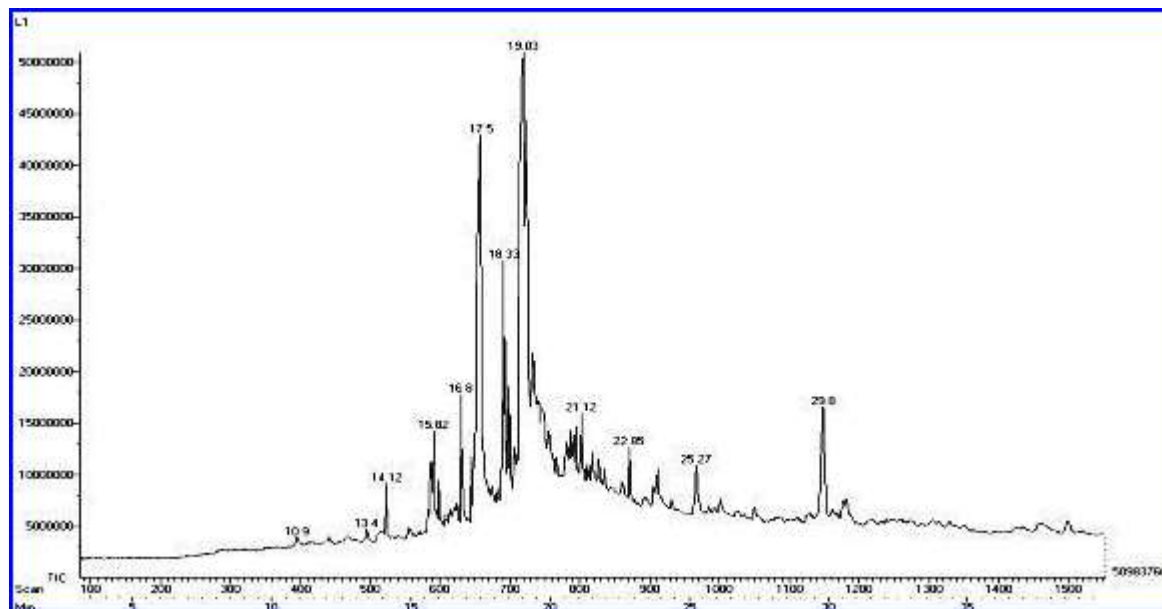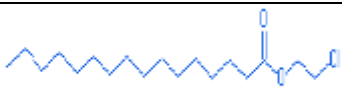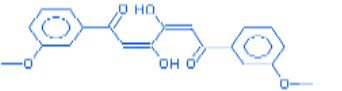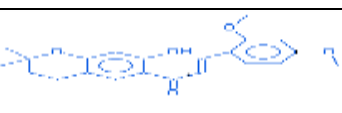

Fig. 3 GC-MS Spectrum of leaf extract of *Terminalia arjuna*

Table 2. Phytochemicals present in the leaf extract of *Terminalia arjuna*

PEAK	R. TIME (R_t)	CHEMICAL FORMULA	COMPOUND	STRUCTURE
1	10.9	$C_8H_{16}O$	Hexanal,4,4-dimmethyl-	
2	13.4	$C_9H_{12}O$	Phenol,4-propyl-	
3	14.12	$C_{11}H_{14}$	Benzene,[1-methylenebutyl]-	
4	15.82	$(CH_4NH_2)_2$	Benzidine	
5	16.8	$C_{15}H_{16}O_2$	Flavone	

6	17.5	C$_{19}$H$_{36}$O	Estra-1,3,5(10)-trien-17á-0l	
7	18.33	C$_{19}$H$_{36}$O$_2$	10-Octadecanoic acid, methyl ester	
8	19.03	C$_{18}$H$_{34}$O$_2$	Octadec-9-enoic acid	
9	21.12	C$_{18}$H$_{35}$ClO$_2$	2-chloroethyl palmitate	
10	22.85	C$_{22}$H$_{38}$O$_3$	2,5-furandione, dihydro-3-isooctadecyl-	
11	25.27	C$_{21}$H$_{40}$O$_4$	1,13,Dioxacyclotetracos ane -2,14-dione	
12	29.8	C$_{22}$H$_{24}$O$_5$	2,2-dimethyl-7-hydroxy-6-[2,4 dimethoxycinnamoyl] chroane	

III. UV-Vis spectroscopic analysis: The different phytoconstituents present in medicinal plants, like, flavonoids, alkaloids, phenols, tannins, terpenes and the like give specific distinctiveness and properties to plants. UV-Vis spectroscopic technique is simple, cost-effective and rapid test for detecting these phytoconstituents. Molecules undergo electronic transitions in the visible and UV region of the electromagnetic spectrum is the basis for this technique.

The UV-VIS spectrum of the leaf extract has absorption band at 313nm and broad peaks at 230-250nm (Fig. 4). These absorption bands are characteristic for flavonoids and its derivatives. This has been reported by Mamta *et al* for the plant *Acorus calamus* (22). The flavonoids spectra typically consists of two absorption maxima in the ranges 240-285 nm (band II) and 300-380 nm (band I) (23, 24). The precise position and relative intensities of recorded maxima in the spectrum indicate that flavonoid is present in leaf extract of *Terminalia arjuna*. These correspond to n-σ* transition. Saturated compounds containing one heteroatom with unshared pair of electrons like O, N, S and halogens are capable of n-σ* transitions. Also the appearance of one or more peaks in the region from 200 – 400 nm is a clear indication of the presence of unsaturated groups and heteroatoms such as S, N and O (25).
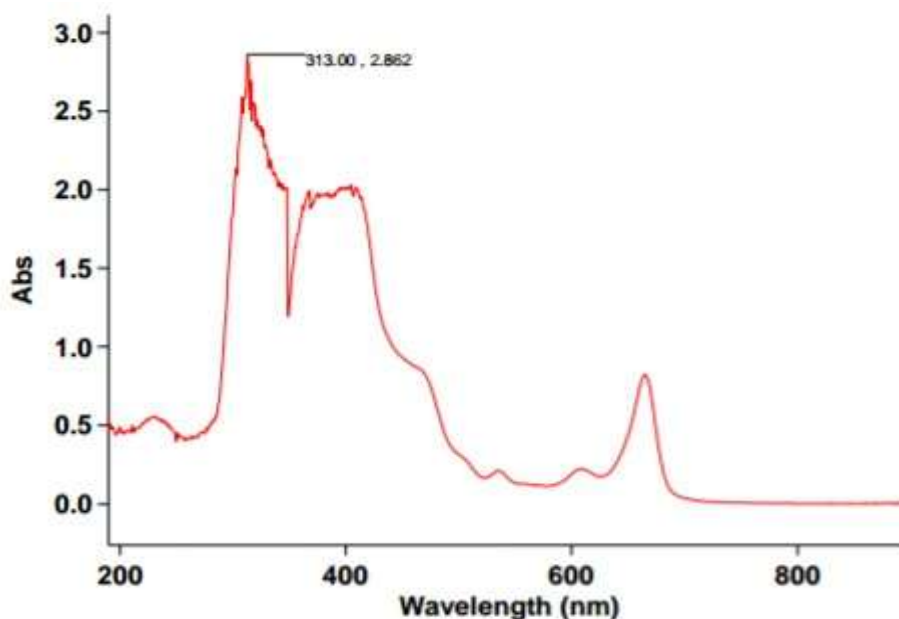
Fig. 4 UV-Vis spectrum for leaf extract of *Terminalia arjuna*

IV. Anti-microbial studies on the leaf extract of *Terminalia arjuna*:

The clinical efficacy of many existing chemotherapeutic agents is being threatened by the emergence of multidrug-resistant pathogens. Bacteria naturally develop resistance to antimicrobial drugs. In recent years, the overuse and misuse of antibiotics has caused a growing number of staphylococcus bacteria to evolve into disease causing "superbugs". These superbugs are resistant to any potent drugs like methicillin, vancomycin [27]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several plants for their potential antimicrobial activity and development of new antimicrobials by drug companies. Herbs are staging a comeback all over the globe. According to WHO, 80% of the world's population relies on plant-based traditional medicines for their primary healthcare needs [28].

A wide variety of medicinal plants used traditionally have not yet been systematically investigated against various microbial pathogens. Literature study reveals that a few studies on antibacterial and antifungal activity of various parts of *Terminalia arjuna* plant, such as bark has been carried out [29]. However, antimicrobial studies of leaf extract of the plant are lacking. The present study was carried out to validate the antimicrobial potential of *T. arjuna* leaves against a few bacterial and fungal pathogens.

Preparation of inoculum: Stock cultures were maintained at 4°C on Nutrient agar Slant. Active cultures for experiments were prepared by transferring a loop full of culture from the stock cultures into the test tubes containing nutrient broth, that were incubated for 24 hours at 37ºC. The Assay was performed by agar disc diffusion method [30].

In present study, an attempt has been made to investigate the antimicrobial activity of *Terminalia arjuna* extract against two species of fungi *Aspergillus niger and Candida albicans* and four strains bacteria, *Escherichia coli* and *pseudomonas aeruginosa* (gram negative), *streptococcus and staplylococcus aureus* (gram positive). The results presented in Tables 3 and 4 revealed that the leaf extract displayed potential antifungal activity against the tested fungal pathogens, *Aspergillus niger* and *Candida albicans. Terminalia arjuna* leaf extract exhibited good antimicrobial activity against *Escherichia coli, streptococcus, staplylococcus aureus,* and *pseudomonas aeruginosa.* Study of the zone of inhibition showed that the leaf extract was least effective against *E.Coli* bacteria whereas it showed considerable action against other three bacterial pathogens.

Aspergillus niger   *Candida albicans*

Fig. 5 Zone of inhibition for leaf extract of *Terminalia arjuna* for *Aspergillus niger* and *Candida albicans*

Table 3.Antifungal activity for leaf extract of *Terminalia arjuna:*

Organism	Zone of inhibition (mm) Concentration (mg/ml)			Antibiotic Amphotericin-B (1mg/ml)
	1	**0.75**	**0.5**	
Aspergillus niger	7	-	7	22
Candida albicans	15	-	-	15

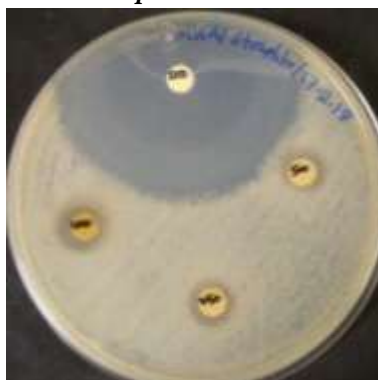*Escherichia coli*   *Pseudomonas*

Streptococcous   *Staphylococcus*

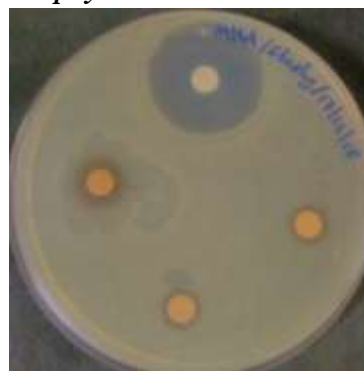**Fig. 6 Zone of inhibition for leaf extract of *Terminalia arjuna* for *Eschericha coli, Pseudomonas aeruginosa, Streptococcus* and *Staphylococcus aureus***

Table 4. Antibacterial activity for leaf extract of *Terminalia arjuna*

Organism	Zone of Inhibition(mm) Concentration(µg/ml)			Antibiotic Ampicillin
	1000	750	500	
Escherichia coli	11	9	8	25
Staphylococcus	14	10	8	29
Streptococcous	15	11	11	40
Pseudomonas	14	10	8	33

Conclusion

A thorough spectroscopic analysis of the ethanolic extract of leaf Terminalia arjuna has been carried out using tools like FTIR, GC-MS and UV-Visible techniques in this work. The various phytochemicals that could be used as effective therapeutic agents have been identified using these methods. Not much work has been reported on the phytochemical screening or therapeutic activity of leaf extract of the plant, though the bark has been used as potent cardiac medicine for many years. So in this study the potential use of leaf extract of Terminalia arjuna as medicine has been studied. The various functional groups and important chromophores present in the leaf extract have been identified using the FTIR and UV-Visible spectra respectively. Further the structures of the major phytochemicals present in the leaf were obtained from the GC-MS spectrum of the extract. The GC-MS spectrum revealed the presence of important esters and flavonoids that are known to possess anti-cancer and other potential therapeutic properties. So, further research is under progress to study the anti-cancer property of leaf-extract of the plant. Also the anti-fungal and anti-bacterial activity of the extract against common fungal and bacterial pathogens has been studied and it was found that ethanolic extract of *Terminalia arjuna* leaf had significant anti-microbial properties.

Conflict of interest : The author declares that there is no conflict of interest.

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