

Phytochemical And Pharmacological Evaluation Of *Tylophora Indica* (Burm. F.) Merr. Extract For Antioxidant Activity.

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ABSTRACT

The present study, “*Phytochemical and Pharmacological Evaluation of Tylophora indica* (Burm. f.) Merr. Extract,” focuses on the comprehensive investigation of the plant’s bioactive constituents and antioxidant potential. The aerial parts of *Tylophora indica* were subjected to solvent extraction using petroleum ether, ethanol, and aqueous media, with ethanolic extract showing the highest yield (16.67%). Qualitative and quantitative phytochemical analyses revealed the presence of flavonoids, phenolics, alkaloids, terpenoids, saponins, and steroids, indicating a rich phytochemical profile. Spectroscopic characterization through ATR-FTIR, GC-MS, and HPLC confirmed the presence of multiple bioactive compounds associated with therapeutic properties. The antioxidant efficacy was evaluated using DPPH, ABTS, and hydroxyl radical scavenging assays. The ethanolic extract exhibited significant radical scavenging activity with inhibition rates of 93.48% (DPPH), 43.72% (ABTS), and 68.47% (hydroxyl), comparable to standard ascorbic acid. These findings suggest that *Tylophora indica* is a potent natural source of antioxidants, potentially beneficial in preventing oxidative stress-related disorders. Overall, this study provides a scientific basis for the traditional use of *Tylophora indica* and highlights its potential for future development as a natural antioxidant and therapeutic agent.

INTRODUCTION

Throughout human history, herbal medicine has been utilised by all societies, making it the most ancient and widely used form of healthcare. The basis for ancient medicine around the world is plants and herbs, which are among the most vital processes of life on Earth. Of approximately 350,000 varieties of living plants, including ferns, bryophytes, & seed plants, 287,655 had been identified as of 2004. Biology medicine, phytomedicine, or phytotherapy are additional names for herbal medicine (HM), which is the practice of treating botanical materials, herbal preparations, and completed herbal products that contain active compounds derived from plant or other substances¹. Botanical medicine, phytomedicine, and phytotherapy are other terms frequently used to describe herbal medicine. Herbal medicine makes use of bark, flowers, fruits, seeds, berries, roots, leaves, and even the complete plant. Man mostly relied on

raw plant material to maintain health and cure illnesses prior to the discovery of aspirin, which is derived from the *Spiraea*, *Ulm* plant, as the Greek physician Hippocrates advised for pain and fever, as well as for fever as well as swelling in Egyptian papyri ².

Traditional herbal treatment has long been utilized for medicinal purposes and is widely acknowledged as safe and beneficial. One active ingredient derived from plant material, which can be obtained from plant extracts or synthesised to mimic naturally occurring plant compounds, is present in about one-third of all prescription drugs today. A large number of today's commonly used drugs in developing nations have herbal origins. There is a long olden times of use aimed at many of the medications that are being recommended to physicians as herbal remedies. The World Health Organisation estimates that about 25% of medications that are currently prescribed in the US

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

are thought to have plant origins. More than 120 active substances that are derived from higher plants are currently available³.

Both illness prevention and treatment are achieved with herbal remedies. Herbal remedies have long been referred to as "the people's medicines" due toward their ease of obtainability, safety, and effortlessness of production. It has long been known that herbal remedies can be used to treat illnesses in addition to conventional medications⁴.

Herbal treatments are still commonly utilized in many economically prosperous Asian nations, such as Japan, China, Thailand, and South Korea, despite the easy access to allopathic pharmaceuticals and state-of-the-art modern healthcare facilities in many countries today. Sixty to seventy percent of allopathic doctors prescribe Kampo therapies, which are mostly herbal medicines, to their patients in Japan. In Malaysia, where traditional forms of these therapies are easily accessible, traditional forms of Malay, Chinese, and Indian medicines—all of which are essentially made from herbs and herbal ingredients—are widely utilised. Herbal medicines are used to treat an estimated 200 million people annually, or roughly 40% of all patients in China who receive medical care⁵.

In recent times, there has been a better effort on finding pharmaceuticals and nutritional supplements made from plants due to the growing popularity of herbal medications and their increasing consumption worldwide. Practitioners in the disciplines of pharmacognosy, microbiology, botany, chemistry of natural products are scouring the globe for phytochemicals and plant-based leads that could be used in the manufacture of medications for a range of diseases⁶.

Status of herbal medicines

The WHO first acknowledged the benefits of herbal medicine and conventional medical practitioners in 1978. The World Health Assembly has released several resolutions pertaining to goals, policies, and initiatives associated with herbal medicine. The WHO's guidelines for evaluating herbal medicine were accepted at the VIth International Conference of Drug Regulatory Authorities that year, which took

place in Ottawa. The three primary parts of WHO guidelines are stability (shelf life), quality evaluation (raw plant materials or plant extract and final product), and safety assessment.

Preclinical, clinical, and documentation of customary usage research are all included in the evaluation of efficacy. Most of the world's traditional medicine needs to be administered, regulated, and managed with a great deal of time and effort⁷.

FUTURE ASPECTS

There might be even more uses for herbal medicine as a form of treatment. The World Health Organisation (WHO) has expressed a strong desire to compile information on the medicinal plant usage of indigenous peoples worldwide. More underprivileged countries than before are working harder than before to compile ethno therapeutic data on HM. If systematic signal supports the claims made by tribal therapists regarding Indian plants, then more research has been done in this area. After a thorough scientific review and dissemination of these regional ethno-medical formulations, people will have a greater understanding of medicinal therapy that works and better health outcomes.

Plant species, including leaves, nonvascular plant, and seed plants, are estimated to number 350,000; as of 2004, 287,655 of these species had been identified. Only a small percentage of them between 1 and 10% are fed to humans and other animals.

Before it is lost forever, the traditional knowledge system needs to be researched, documented, conserved, and applied for the benefit of all people. A detailed plan and the participation of the local community are necessary for this. The Associated Chambers of Commerce and Industry of India (ASSOCHAM), the herbal industry's current market size of Rs7,500 crore (Rs75 billion) will double to Rs15,000 crore by 2015 as a result of the business has maintained an annualized growth rate of more than 20%. According to a 2015 ASSOCHAM study on the global herbal industry and market, India is recognized for its abundance of medicinal herbs and traditional wisdom, with its share exceeding⁸.

ANTI- OXIDANTS



It seems improbable that a high dose of a single or several antioxidants, such as β -carotene or vitamins C and E, would help protect all organs, cells, and tissues against oxidative stress while maintaining all of the beneficial and normal functions that ROS provide. Antioxidant supplements have, in fact, often had little effect as well as had detrimental effects on health. According to recent meta-analyses and reviews, there is no benefit to taking supplements of vitamin C, the antioxidant vitamin E, or β -carotene. Testing the potential health benefits of foods high in antioxidants, which typically contain a variety of antioxidants chosen by means of plant evolution to shield all plant cell components from oxidative damage, is an alternate and much more probable antioxidant strategy for evaluating defence against oxidative effects and related diseases. This is particularly important for spices and herbs. The purpose of this chapter is to discuss oxidative stress, related diseases, and the potential use of antioxidants found in herbs and spices in daily life. After providing a brief introduction to ROS and their role in both oxidative stress and normal physiology, we present evidence showing that the most advantageous dietary source of antioxidants yet identified is found in herbs and spices. We discuss the potential role of antioxidant herbs and spices in oxidative stress as we wrap up the chapter⁹.

REACTIVE OXYGEN ROLE IN CELL SIGNALLING.

Cells must sense their immediate surroundings and modify their activity to fit their microenvironment in order to survive. Cell signalling is responsible for this. By controlling the movement of protein along the pathway, a fundamental signalling pathway spreads a signal across the cell. A "second messenger" or "mediator" is a molecule that either activates or inhibits a signalling cascade stage. Throughout signalling pathways, ROS functions have been reported at numerous locations. ROS particle had been recognized as both the "effector," the last stage in the signalling process, and the "initiator," the initial stimulus that starts the chain reactions of a signalling route. Moreover, ROS can function as a molecule that sends an indication or by enhancing a pathway step halfway between the start and conclusion of the signalling pathway. ROS can be recognized as an

intermediate in the particular pathway in both cases. Fortunately, ROS must be generated when and when needed, detected by a system, and quickly removed to stop the signal from continuing in order to function as signalling intermediaries¹⁰.

REACTIVE OXYGEN SPECIES PRODUCTION

Following the reduction of oxygen to water, ROS molecules are created. Superoxide is created when one electron combines with oxygen, and hydrogen peroxide is created when more reduction occurs. ROS can be produced by both endogenous and external stresses, including ultraviolet (UV) radiation, chemotherapy, environmental contaminants, and physical activity. Nicotinamide is A (NADPH), oxidases that including NOX or a dual oxidase [DUOX], nitric oxide (NOS) synthesis (NOS), the xanthine oxidase, as well as the chain of electron transport in mitochondria are some of the enzymes that cause the deliberate generation of ROS in different cellular compartments.

The seven NADPH oxidases are DUOX1 &

DUOX2 and NOX1-5. These transmembrane proteins convert hydrogen into superoxide or peroxide. The oxidases NOX1 through NOX5 move electrons in NADPH onto the oxygen through the membrane to form superoxide. In addition to transmitting an electron from NADPH, the calcium-dependent enzymes DUOX1-2 directly produce hydrogen peroxide from a peroxidase-like subunit on the outer side of the membrane. The cellular division, towards activators, and associated subunits of the enzymes also differ. It has been demonstrated that NOX synthesis is stimulated by growth hormones, cytokines and vitamin D.

Historically, it was thought that ROS were produced by mitochondria as an undesirable consequence of energy generation within an electron transport chain. But the mitochondria themselves are also capable of generating ROS. This occurs, at least in part, because NO inhibits cytochrome c oxidase, which increases the creation of superoxide while maintaining the same level of energy production. Oxygen is converted to hydrogen peroxide by mitochondrial superoxide dismutase, which can pass across membranes and interact with cytoplasm¹¹.



EXTRACTION

Plant components (coarse or powders) were immersed in a solvent-filled, lidded container for a minimum of three days and agitated repeatedly. For the release of the soluble chemicals during extraction, a plant's wall of cells was designed to weaken and crack. The mixture is crushed or purified three days later.

Similarly to maceration, infusion and decoction entail immersing the substances in hot or cold water. In contrast with maceration and infusion, decoction

frequently yields a greater quantity of oilsoluble chemicals and is only suitable for extraction heat-stable compounds from hard plant substances (such as roots and bark). Infusions necessitate a shorter soaking time than decoctions, which involve boiling the sample in a particular amount of water for a certain amount of time. Percolation, which uses particular tools known as a percolator, employs the same core notion but takes a different technique. The percolating device is filled with drying powdered samples, then hot water is added and the combination settled for two hours. For more concentrate extracts, the percolation method is often repeated till the extraction ends at a slow rate (for example, 6 drops per minute)¹²⁻¹³.

PHARMACEUTICAL ANALYTICAL TECHNIQUES

Spectral methods:

Where we study light absorption or emission by the compounds depends on Characteristics of drugs & pharmaceuticals, viz., UV-Visible spectroscopy, IR spectroscopy, NMR spectroscopy and

Fluorimetry.

Chromatographic methods:

Thin layer chromatography (TLC), higher pressure liquid chromatography (HPLC), gas chromatography (GC), high pressure thin layer chromatography (HPTLC), and ultra pressure liquid chromatography (UPLC) are the methods where we investigate the differences in affinity or partition coefficients of medications and pharmaceuticals.

Electro-analytical techniques:

Where we study Electro – chemical properties like conductance, current, potential and resistance of drug and pharmaceuticals, Via, Potentiometry, conductometry, polarography and Amperometry.

Biological and microbiological methods:

Where we use either animals or Microorganisms for analysis of drugs and pharmaceuticals, viz., biological assay of some vitamins, vaccines, antibiotics and vitamins.

Radio analytical methods:

These are used in estimation of hormones, drugs and pharmaceuticals (Radio immunoassay).

Thermo analytical methods:

These methods are used to analyze the changes of some physical & chemical assets of drugs and pharmaceuticals with respective of time or temperature for qualitative and quantitative analysis. DTA (Differential thermal analysis), DSC (Differential scanning calorimetry), TMA (Thermal mechanical analysis) and TGA (Thermo gravimetric analysis) are some of the thermo analytical methods¹⁴⁻¹⁵.

FOURIER TRANSFORM INFRARED (FT-IR)

To overcome the drawbacks of dispersive devices, Spectrometer was created. The scanning process's slowness was the main problem. Instead of monitoring each thermal frequency independently, a system that could sense them all simultaneously was needed. An exceedingly simple optical device called an interferometer was used to create a solution. Every infrared frequency is "encoded" into a unique signal that is produced by the interferometer. It is possible to monitor the signal quickly, typically in less than a second. This reduces the amount of time needed for each sample from many minutes to a few seconds. The beam splitter found in the majority of interferometers divides the incoming the infrared beam into 2 visible beams. . A single-celled light bounces off a stationary, even mirror. A convex mirror that is connected to a system that allows it to



move just a few millimetres to the light splitter reflected the other beam. At the light splitter, the two beams recombined after reflecting off their respective mirrors. The message that emerges from an interferometer is the result of both of these beams "interfering" with each other because one beam follows a predetermined route while the other changes continuously as the mirror rotates. The resultant signal is called an interferogram, which has the amazing ability to contain information on each infrared frequency emitted at every single point of data (a consequence of the mirror's rotational position).

This indicates that each of the frequencies are measured simultaneously when the interferogram is being measured.

Consequently, the interferometer enables incredibly fast measurements. The detected interferogram signals cannot be assessed instantly, even though an analyst needs a spectrum of frequency (a representation of the signal's strength at each specific frequency) to make a conclusion. A technique for "decoding" particular frequencies is required. The Fourier transform is a well-known mathematical technique that can be used to do this. The computer does this change, after which it gives the user the relevant spectrum data for examination.

Sample Analysis Process

- 1. Source:** Infrared energy is emitted by a bright black-body source. Through an aperture, this beam controls the quantity of heat that is transferred to the materials (and, ultimately, the detector).
- 2. The light:** that enters an interferometer, wherein "spectral encoding" takes place, is known as the spectrometer. After that, the interferogram signal exits the interferometer.
- 3. The Sample:** Depending on the type of analysis being done, the beam either passes through the sample compartment or bounces off the sample's exterior. This is where specific energy frequencies that are specific to the material under study are absorbed.
- 4. The Detectors:** The detector the final measurement are the ultimate targets of the light source. The particular interferogram signal is captured by specially designed detectors.
- 5. The computer:** After the Fourier analysis is completed, the measured signal is digitalized and sent to the computer. The viewer is then presented with the completed infrared spectrum for review and additional editing.

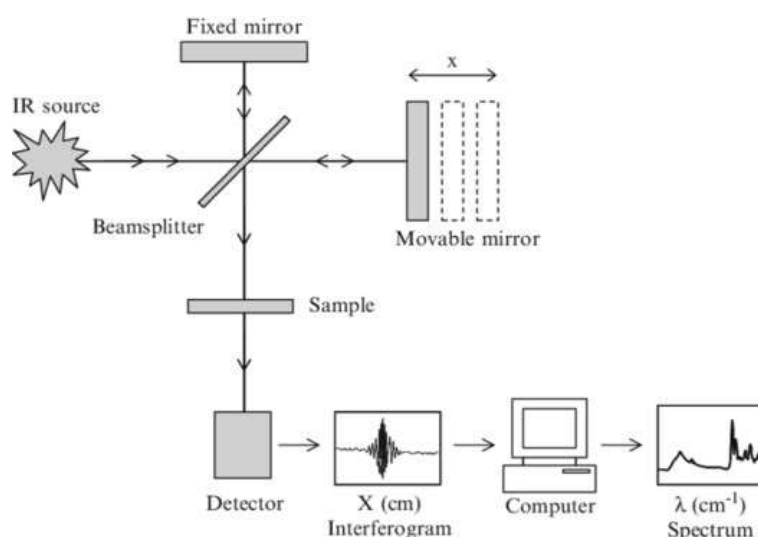


Fig.1. FTIR

These benefits, together with others, make FT-IR measurements exceedingly accurate and reproducible. It is therefore a very dependable method for positively

detecting practically any material. Even the smallest contaminants can be detected thanks to the level of sensitivity benefits. Because of this, FT-IR is a useful

tool for quality assurance and control applications, such as assessing an unknown contaminant or comparing batch to quality standards. Additionally, the practical application of visible light for quantitative assessment has been greatly increased by the sensitivity and accuracy of FT-IR detectors as well as a variety of software methods. Quantitative methods can be included into routine analysis processes and are simple to develop and calibrate. thereby, the (FT-IR) approach has provided major practical benefits to infrared spectroscopy. It has enabled the creation of numerous new sampling approaches aimed to address difficult problems that were previously impossible to solve with older technologies. It has almost eliminated the limitations on infrared analysis¹⁶⁻¹⁸.

GAS CHROMATOGRAPHY

"It involves employing a mobile gaseous phase to separate the component or components from the provided crude medicament. After being vaporized, the sample is placed into the chromatography column's tip. The sample is transported by the inert, gaseous mobile phase as it passes through the column. A stationary form of liquid that has been adsorbed on the outermost layer of an inert solid is present in the column¹⁹.

Two major types:

1. Gas-solid chromatography: A solid serves as the stationary phase, while a gas serves as the mobile phase. used for the separation of low molecular gases, including nitrogen oxides, H₂S, CS₂, CO₂, rarer gases, and air components.

2. Gas-liquid chromatography: A liquid serves as the stationary stage and a gas as the traveling phase. retained as a solid inert on its surface through chemical bonding or adsorption. Four guiding principles: The idea behind GC's separation is "partition."

The mixture of the components that need to be separated is turned into vapor and mixed with the gaseous in nature mobile phase.

In the stationary state, the more easily soluble component elutes later and travels more slowly. The

stationary phase's less soluble component elutes first and travels more quickly.

The requirements for partition coefficients are different for each component. Thus, the partition coefficient is used to separate the components.

"The proportion of the soluble of an element spread across 2 liquids that are insoluble at a constant temperature" is the definition of the partition coefficient²⁰.

INSTRUMENTATION:

- Helium (common), N₂, H₂, and Argon are carrier gases.
- Port for injecting samples: micro syringe
- Columns

DETECTORS:

- Thermal conductivity (TCD)
- Electron capture detector (ECD)
- Flame Ionization detector (FID)
- Flame photometric (FPD)

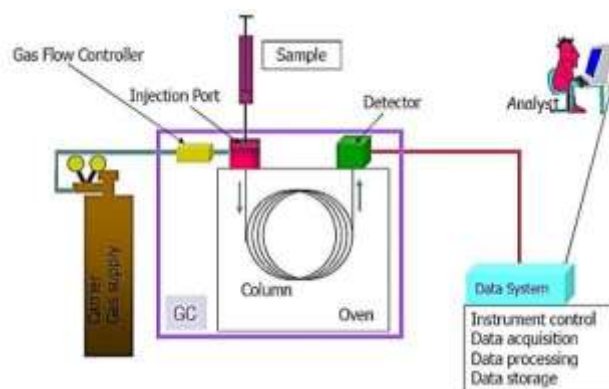


Fig.2. Gas Chromatography

PLANT PROFILE

Tylophora Indica (Burm.f.) Merr

Scientific synonyms

Tylophora asthmatica (Roxb.) Wight & Arn.

Family: Apocynaceae



Regional synonyms

Tamil	: Nancaruppan, Naippalai
English	: Indian Sarsaparilla
Telugu	: Kakapala
Kannada	: Adumuttada Gida
Marathi	: Khadari, Pithakaadi
Hindi	: Antamul and Janglipikvam
Malayalam	: Vallipaala
Sanskrit	: Arkaparni and Lataksiri

Plant taxonomy

Kingdom	: Plantae
Phylum	: Tracheophyta
Class	: Magnoliopsida
Order	: Gentianales
Family	: Apocynaceae
Genus	: <i>Tylophora</i>
Species	: <i>Tylophora indica</i> (Burm.f.) Merr

Habitat

India, Sri Lanka

Parts used

Aerial parts

Geographical Distribution

Tylophora indica is found in several places, including India, Sri Lanka, Bangladesh, Malaysia, and southeast Asia. Throughout India, the plant can be found in thickets on plains, hills, and woods. It can be found in Tamil Nadu (Vellore, Tiruvannamalai, and Salem, a Tirunelveli), Karnataka, Kerala (all regions), Odisha, and Andhra Pradesh. It likes soil that drains well and has a lot of humus.

Plant Description

With oval in order elliptic-oblong leaves ranging from 3–10 cm in length and 1.5–7 cm broad, it is a branched climber or shrub that grows to a height of 1.5 meters. Long, meaty roots with longitudinally fissured, light brown, corky bark. Within axillary umbellate cymes, the small, 1–1.5 cm-wide flowers are grouped in 2–3 flowered fascicles. Almost to the root, the calyx is divided lanceolately and acutely; the exterior is very hairy. The color of the corolla is either greenish yellow and greenish purple. Lobes are oblong and pointed. The end output is a fine macro levels, striate, and glabrous follicle which can be up to 7×1 cm in size, ovoid, lanceolate, and tapering at the apex. seeds that are 0.6–0.8 x 0.3–0.4 cm long²¹.



Fig.3. *Tylophora indica* plant

Pharmacological/Medicinal Importance

Tylophora indica (Burm.f.) Merr. contains a variety of pharmacologically and medicinally important chemical elements, including alkaloids, which tylophorine, tylophorinidine, sterols and septicine, flavonoids, and tannins. This has been recognized as a significant plant in both traditional and contemporary drug development domains due to its numerous medicinal applications. Human use of it is safe. The aerial parts of the plant, including the stem, roots, and leaves, have long been used as medicines. *T. indica* has many properties, including as hepatoprotective, diuretic, immunosuppressive, anticancer, antifeedant, antibacterial, antifungal, and antiamebic activities²².

Selection of Model Drug (Plant)

The choice of a medicinal plant with antioxidant properties is discussed with tribal medical

professionals regarding the traditional usage of the entire plant. Therefore, the pharmacological assessment of the antioxidant activity in the chosen natural product (*Tylophora indica*) is part of the current investigation²³.

Procurement, Collection and Authentication of Plant

Fresh plant material of *Tylophora indica* (Burm.f) Merr. was acquired from the Xavier Research Foundation in Palamkottai, Tamilnadu. In the Tamil Nadu district of Tirunelveli, the herb *Tylophora indica* (Burm.f.) merr. was collected from the Kavalkinaru neighborhood. The plant was recognized and botanically confirmed by Mr. V. Chelladurai, a botanist from the Central Council for Research in Ayurvedic & Siddha (Govt. of India), Research Office Botany (Scientist -C)²⁴.

Extraction of the Plant Petroleum Ether Extract of *Tylophora indica*

Using a soxhlet unit, 250g of the processed whole herbal plant was extracted over the course of 72 hours at a temperature between 60 and 80 °C using one litre of petroleum ether. The filtrate was gathered, dried under low pressure, and the powdered extract was kept dry in a desiccator until needed again. Calculations and records were made regarding the extractive value and percentage yield of the extract.

Ethanollic Plant Extract in *Tylophora indica*

Following the research of the petroleum extract, the leftover material was dried and then extracted using two litres of 99% V/V ethanol through a continuous percolation process using a soxhlet unit for 72 hours at a temperature between 75 and 80 °C. The filtrate was gathered, dried under low pressure, and the powdered extract was kept dry in a desiccator until needed again. A calculation and recording of the extractive value and extraction yield percentage were made.

Aqueous Plant Extract of *Tylophora Indica*

After the ethanolic extract was prepared, the leftover plant was dried and then extracted using two litres of

water and 0.25% ethanol through a 96 hour percolation process. A muslin cloth was then used to filter the extract. After drying and condensing the filtrate under low pressure, the dry powder extract was stored inside a desiccator until it was needed again. A calculation and recording of the extractive value and extraction yield percentage were made²⁵.



Fig.4. Continuous hot percolation method for *Tylophora indica*

Percentage yield of plant

The weight of the manufactured extract that was dried and the weight of the dried plant samples were used to compute the percentage yield.

PRELIMINARY TESTS

To identify the different active ingredients, qualitative tests were performed on all *Tylophora indica* whole plant extracts.

TEST FOR ALKALOIDS:

- a) **Dragendorff's test:** 5 ml of purified water was added to 2 mg each of the extracts and 2 M HCL were added until an acids reaction happened. Dragendorff's reagent (1 ml) was added to this. The presence of alkaloids is indicated by the production of an oranges or orangered precipitate.
- b) **Hager's test:** A few droplets of Hager's solution were applied to two mg of the extracted in a test

tube. The actual presence of alkaloids is confirmed by the production of a yellow precipitate.

- c) **Wagner's test:** a few droplets of Wagner's reagent are used after 2 mg of extract had been acidic with 1.5% v/v hydrochloric acid. Alkaloids are present when a yellow, brown precipitate forms.
- d) **Mayer's test:** Two milligrams of extracts were applied to several drops of Mayer's reagent. When alkaloids are present, a white and pale-yellow precipitate will occur.

TEST FOR CARBOHYDRATES:

- a) **The Anthrone test:** this is involved shaking 2 mg of extracts with 10 ml of water, filtering the resulting filtrate, and then concentrating it. The reagent mixture was added to these two milliliters of anthrone. A presence of carbohydrates is indicated by the production of a green or blue color.
- b) **Benedict's test:** It involves mixing two milligrams of extract with ten milliliters of liquid, filtering the product, and then concentration it. After adding five milliliters of Benedict's solution, it was heated for five minutes. The formation of a precipitate with a brick-red color indicates the presence of carbohydrates.
- c) **Fehling's test:** 10 milliliters of water were agitated with 2 milligrams of extracts, filtered, and the filtrate was then concentrated. After adding 1 milliliter of a mix of equal parts Fehling's solutions A and B, it was brought to a boil for a short while. A red or brick-red precipitate's development suggests that reducing sugar is present.
- d) **Molisch's test:** a filtrate was made by combining 2 mg of extracts with 10 ml of water, then the filtrate had been concentrated. Two drops of newly made α -naphthol 20% alcoholic solution were added. To create a layer underneath the liquid, two milliliters of concentrated sulfuric acid were added. When too much alkali is added, the

reddishviolet ring that indicates the presence of carbs vanishes.

TEST FOR FLAVONOIDS:

- a) **Shinoda's test:** 10 drops of diluted hydrochloric acid were added after 2 mg of extracts had been dissolved in 5 ml of ethanol, which is and then a little piece of magnesium was added. Flavonoids are indicated by the creation of a pink, reddish, or brown color.
- b) **Sulfuric acid test with conc:** orange to red (flavanones), yellow to orange (flavones), and yelloworange (anthocyanins)

TEST FOR GLYCOSIDES:

- a) **Molisch's test:** 10 milliliters of water were agitated with 2 milligrams of extracts, and the filtered and concentrated filtrate was then used. After mixing two to three drops of Molisch's reagent, two milliliters of sulfuric acid concentration were cautiously introduced via the test tube's side. The activity of glycosides is indicated by the appearance of the reddish violet ring.
- #### TEST FOR AMINO ACIDS

The following reagents were added to little amounts of the extracts that had been dissolved in a few milliliters of water.

- a) **Million's reagent:** The level of amino acids is indicated by the red hue.
- b) **Ninhydrin reagent:** The occurrence of amino acids is indicated by the purple hue.

TEST FOR SAPONINS:

- a) **Foam test:** A small amount of a solution of sodium bicarbonate was put to a test tube that held around 5 ml of extracts. After giving the test tube a good shake, it was left for three minutes. When saponins are present, a froth that resembles honeycomb forms.

Measure for tannins.

The following reagents were used to test for a presence of tannins and phenolic compounds after small amounts of each extract were consumed separately in water.



a) Violet-colored diluted ferric chloride solution (5%).

Determine for phenolic substances

After consuming small quantities of each extract individually in water, the following chemicals were employed to test for the presence of tannin and phenolic compounds.

a) White precipitate, a 1% gelatin solution with 10% sodium chloride.

b) Test 10% lead acetate solution - white precipitate.

TEST FOR TERPENOIDS:

a) **Salkowski test:** 5 ml of extract, 2 ml of chloroform, and 3 ml of concentrated sulfuric acid were carefully added to create a layer. Terpenoids are indicated by a reddish-brown color.

Investigate for steroids.

a) **Liebermann-Burchard's test:** 1 milliliter of concentrated sulfuric acid was applied along the test tube's sides after 2 milligrams of dry extracts had been mixed with acetic anhydride, brought to a boil, and cooled. The development of a green hue signifies the existence of steroids.

b) **Salkowski Reaction:** After shaking 2 mg of dried extracts with chloroform, sulfuric acid was gradually introduced by the test tube's sides to the chloroform layer. A presence of steroids was detected by the creation of a red color²⁶.

ANTI-OXIDANT ACTIVITY

Determination of Antioxidant Activity of *Tylophora indica* (burm. F.) Merr. by DPPH Assay²⁷

Using 1, 1-diphenyl-2-picryl hydrazyl (DPPH), the free radical removal capacity of several leaf extracts of the *Tylophora indica* (burm. F.) Merr. species was assessed. In short, 0.004 g of DPPH crystal solid was dissolved into 100 mL of analytical-grade methanol to create a 0.1 mM solution containing DPPH, which was then kept at 4 °C. To create 400 µg/mL stock solutions, 4 mg of plant extract was immersed in 10 mL of methanol. The necessary higher concentrations (50, 100, 150, 200, 250, and 300 µg/mL) were then

prepared by serial dilution with methanol. Three milliliters of DPPH solution were added to each test tube containing two milliliters of plant extract solutions from each concentration. A UV-Vis

Spectrophotometer was used to measure the an abs around 517 nm following a 30minute dark incubation period.

Ascorbic acid was the reference standard chemical in use. 2 mg of ascorbic acid was dissolved in 2.5 mL of water that was distilled to create a stock solution with 800 µg/mL. Next, several concentrated solutions (50, 100, 150, 200, 250, and 300 µg/mL) were produced for serial dilution. The blanks for the corresponding extracts were distilled water, methanol, and ethanol. As a control, 100 µL of ethanol for the ethanol extraction and 100 µL of water for the aqueous extract were combined with 3 milliliters of 0.1 mM DPPH. Every determination was made three times.

Plotting the percentage of inhibition against concentration allowed for the calculation of IC₅₀ values.

$$\text{DPPH \% Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100$$

Where,

A_{control} - mixture of ethanol / water

DPPH -solution,

A_{sample} - sample extract

Determination of Antioxidant Activity of *Tylophora indica* (burm. F.) Merr. by ABTS Assay²⁸

The method employed in the prior study was applied to the ABTS assay. 2.45 mM persulfate of potassium and 7 mM ABTS solution in stock were combined in equal amounts to create the ABTS radical cation. In short, 0.360 g of ABTS, which salt was dissolved in 100 mL of water that was distilled to create a 7 mM ABTS solution. 0.066 g of salt was dissolved in 100 mL of distilled water to create a 2.45 mM potassium persulfate. Next, 10 mL of 7 mM ABTS solution was then added and 10 mL of 2.45 mM potassium persulfate solution were gently mixed to create an ABTS cation radical solution. The combination was



then allowed to sit at room temperature in the dark for 12 hours until the reaction was finished and its absorbance was stable. Using a UV-Vis Spectrophotometer, the generated radical cation is then further diluted in a 1:1 ratio with ethanol to bring the absorbance value down to 0.700 at 734 nm. After mixing 4000 μL of ABTS⁺ solution with 5 μL , *Tylophora indica* (burm. F.) Merr extract of leaves at concentrations of 50, 100, 150, 200, 250, and 300 $\mu\text{g/mL}$, the mixture was left to stand at room temperature for two hours in the dark. At 734 nm, the absorbance was measured with a UV-Vis

Spectrophotometer. The blanks for the ethanol with aqueous extracts were, respectively, ethanol and water. The control consisted of 10 mL of a mixture of 7 mM ABTS, which 2.45 mM K₂S₂O₈, and 20 mL in ethanol for the ethanol extract and 20 mL of water as the solvent for the aqueous extract.

Each solvent extract's reactivity at different concentrations was contrasted with ascorbic acid's. At least three attempts were made at each measurement. The following formula was used to determine the percentage scavenging of ABTS + radical for various extract and standard levels (50 to 300 $\mu\text{g/mL}$):

$$\frac{A \text{ "control"} - A \text{ "sample"}}{A \text{ "control"}} \times 100 = \text{ABTS \% Scavenging}$$

where A sample is the absorbance of a mixture of test extract/standard and ABTS, and A control is the absorbance of a mixture of 10 milliliters of (7 mM ABTS, 2.45 mM

K₂S₂O₈) and blank solvents. The antioxidant activity leaf extract against ABTS + was noted as IC₅₀.

***Tylophora indica* (burm. F.) Merr. Total Antioxidant Capacity (TAC) measurement using the Phosphomolybdenum Assay²⁹**

Using the phosphomolybdenum assay, the crude extracts' overall antioxidant capacity was assessed. The extract's decrease of Mo(VI) - Mo(V) and the subsequent production of the green phosphorus (Mo(V)) complex at an acidic pH are the basis for the assay. Twenty milliliters of distilled water were mixed with one milliliter of each of 0.6 M of sulfuric acid, 28 mM salt phosphate, and 4 mM ammonium molybdate.

Distilled water was then added to bring the volume up to fifty milliliters. Each test tube included 3 mL of reagent solution, and 0.3 mL of crude extract of *Tylophora indica* (burm. F.) Merr at varying concentrations range from 50 μL to 300 μL were added. For 90 minutes, these tubes were incubated at 95 °C. After the solution had cooled to room temperature, its absorbance at 695 nm was measured against a blank using a UV-VIS spectrophotometer. H₂O and EtOH served as the blanks for the corresponding extracts. 0.3 mL in control and three milliliters of a molybdate solution in 20 milliliters of water were utilized. As a positive reference standard, ascorbic acid was utilized. For every extract, the mean values for three trials were computed. The following formula was used to assess the antioxidant capacity:

$$\frac{A \text{ "sample"} - A \text{ "control"}}{A \text{ "control"}} \times 100 = \text{Antioxidant \%}$$

where A control represents the absorbance for the control and A sample represents the absorption of the sample. The extract concentration below which fifty percent of the inhibition is seen (IC₅₀) was computed and expressed in $\mu\text{g/mL}$.

Determination of Antioxidant Activity of *Tylophora indica* (burm. F.) Merr leave Extracts by Hydrogen Peroxide Scavenging (H₂O₂) Assay³⁰

The procedure was used to determine the extracts' capacity to scavenge hydrogen peroxide. Hydrogen peroxide (two mM) has been prepared into a solution in a phosphate buffer solution with (pH 7.4). In short, solutions of 0.2 M NaOH and a value of M dihydrogen phosphate of potassium were made. Phosphate buffer (pH-7.4) was prepared by adding 50 mL of the potassium dihydrogen phosphate solutions to a 200 mL volumetric flask, followed by 39.1 mL of 0.2 M of sodium hydroxide solution and 200 mL of distilled water. To create the free radicals, 50 milliliters of a solution of phosphate buffer were introduced to an equivalent volume of hydrogen peroxide. The reaction was then allowed to finish for five minutes at room temperature.

The process was performed to find out how well the extracts scavenged hydrogen peroxide. A solution of hydrogen peroxide (two milligrams) has been made in



a solution of phosphate buffer (pH 7.4). In summary, 0.2 M NaOH solutions and a potassium M dihydrogen phosphate value were prepared. 50 mL of potassium dihydrogen phosphate solutions, 39.1 mL of sodium hydroxide solution with a 0.2 M concentration, and 200 mL of distilled water were added to a 200 mL volumetric flask to create phosphate buffer (pH-7.4). The same amount of hydrogen peroxide was mixed with fifty milliliters of a phosphate buffer solution to produce the free radicals. After that, the reaction was let to finish at room temperature for five minutes.

H₂O₂ Scavenging = A BLANK – A SAMPLE / A BLANK

Where,

A blank -absorbance of the blank,

sample -absorbance of a sample or standard.

RESULT AND DISCUSSION

Physical Description and Organoleptic Features of the Plant

The initial stage in identifying herbal plants is to assess the plant's physical description and organoleptic properties. In the end, this improves patient compliance by helping determine patient acceptability traits like color, their nature, odor, and taste.

S. No	Name of the Plant	Colour	Odour
1	<i>Tylophora indica</i>	Greenish pale to yellow	Odourless

Physical report /organoleptic features of the plant

Discussion

The determination of organoleptic properties of selected plant appears greenish pale to yellow in colour with odourless in nature.

Percentage Yield of Plant Extract (*Tylophora Indica*)

Solvent and % yield of plant extract		
Petroleum Ether	Ethanol	Aqueous
13.69%	16.67%	15.40%

Discussion

The percentage yield of herbal plant was obtained by using various solvent used are Petroleum Ether, Ethanol and aqueous solvent. The percentage yield of *Tylophora indica* was found to be 13.69% in petroleum Ether, 16.67% in Ethanol, and 15.40% in aqueous solvent. From the effect of percentage yield of all herbal abstract the ethanolic extract show the highest percentage yield when compared to other extract.

fig.5.Preliminary Phytochemical Test of Entire Plant Part Extract of *Tylophora Indica*

The products obtained after extraction using different solvents system is exposed to different chemical tests for phytochemical test and estimation of the active constituents used in the extracts.





S. No.	Phyto constituents	Pet. Ether fraction	Ethanollic fraction	Aqueous fraction
1	Alkaloids	+	+++	--
2	Amino acids	+	+	--
3	Carbohydrate	+	++	++
4	Terpenoids	--	++	--
5	Flavonoids	--	+++	--
6	Glycosides	+	+	++
7	Phenols	+	+	--
8	Tannins	--	--	--
9	Saponins	--	+	++
10	Steroids	+	++	--
11	Tannins/ Phenolic compounds	+	+	--
12	Triterpenoids	--	+	--

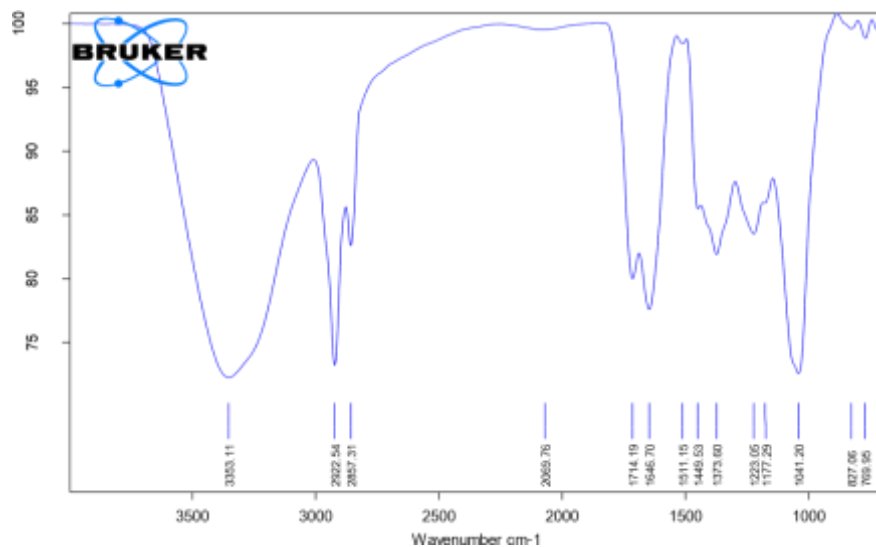
Note: “--” - not, “+” - slight presence, “++” - strong presence, “+++” – More strong presence.

DISCUSSION

The phytochemical properties of a *Tylophora Indica* extract were studied. Petroleum ether, ethanol, & an aqueous solution of the above-mentioned plant

components were among the solvents with increasing polarity that were used to extract the active plant-based elements. Alkaloids, also the amino acids, flavonoids, or steroids, the saponins and other substances were found to be present during the examination. The findings are present in the table.

The analysis of the ATR-FTIR spectrum

Fig.6.Characteristics Peaks for functional group in of *Tylophora indica* plant extract

S.no	Wave number (cm ⁻¹)		Bond and functional group
	Range	Observed peak	
1	1000-650 cm ⁻¹	769, 827	=C- Alkenes
2	1250-1020 cm ⁻¹	1041	C-N aliphatic amines
3	1300-1150 cm ⁻¹	1177, 1223	C-H alkyl halides
4	1500-1400 cm ⁻¹	1449	C-C aromatic
5	1550-1500 cm ⁻¹	1511	N-O nitro compounds
6	1680-1640 cm ⁻¹	1646	-C=C- alkenes
7	1720-1706 cm ⁻¹	1714	C=O carboxylic acid
8	2140-1990 cm ⁻¹	2069	N=C=S isothiocyanate
9	3000-2840 cm ⁻¹	2857, 2922	C-H alkanes
10	3400-3250 cm ⁻¹	3353	O-H alcohol

DISCUSSION

The ATR-FTIR spectroscopy of *Tylophora indica* ethanol extract exhibits various wavelength (cm⁻¹) range such as 769 cm⁻¹, 827 cm⁻¹, 1041 cm⁻¹, 1177 cm⁻¹, 1223 cm⁻¹,

1449 cm⁻¹, 1511 cm⁻¹, 1646 cm⁻¹, 1714 cm⁻¹, 2069 cm⁻¹, 2857 cm⁻¹, 2922 cm⁻¹, 3353 cm⁻¹. It demonstrates the existence of discrete functional category being as alcohol, aliphatic amine, alkanes, alkenes, aromatic etc.

Gas chromatography – Mass spectrometer (GC-MS)

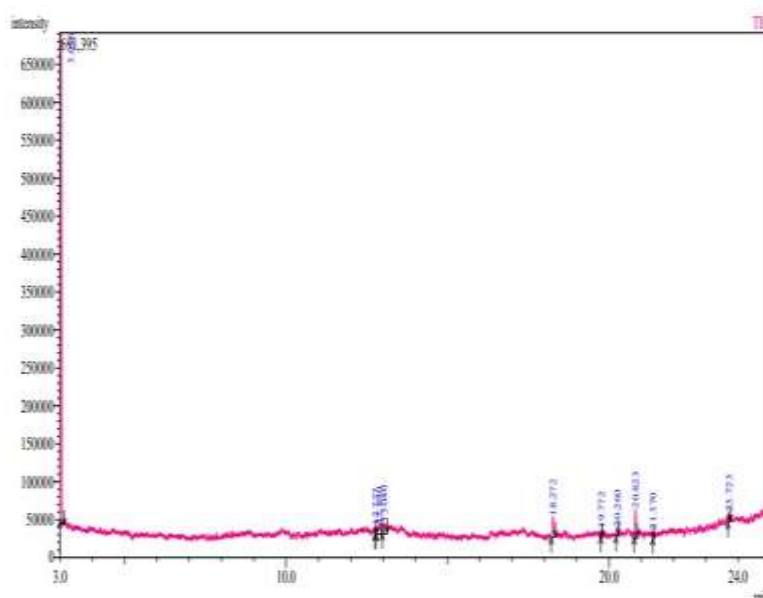


Fig.7. GC-MS analysis of *Tylophora indica* plant ethanol extract

Peak	R. Time	Area	Area %	Height	Height %	Name
1	3.036	1209141	69.92	644746	81.18	Toluene
2	12.777	18321	1.06	11361	1.43	4- {[3-(Furan-2-yl) propyl] carbamoyl} butanoic acid
3	12.890	107305	6.20	11910	1.50	7-(4-Methoxyphenyl)-2-(trifluoromethyl)-[1,2,4]triazolo[1,5a] pyrimidine
4	13.040	95405	5.52	14368	1.81	Spiro[4.5]decane-1,6-dione
5	18.272	86638	5.01	27340	3.44	2-Pentadecanone, 6,10,14-trimethyl-
6	19.772	23133	1.34	11235	1.41	5-Amino-2H-1benzofuran-3-one
7	20.260	28795	1.67	11347	1.43	Benzoic acid, 2-(1oxopropyl)-
8	20.823	113950	6.59	38362	4.83	8-Methylnonanoic acid, ethyl ester, Octadecanoic acid, ethyl ester
9	21.370	12985	0.75	11308	1.42	Oxirane,2,2'(1,4butanediyl)

10	23.723	33707	1.95	12199	1.54	[5,9-Dimethyl-1(3-phenyl-oxiran-2-yl)-deca-4,8dienylindene]-(2-phenyl-aziridin-1-ly)-amine
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GC-MS peak report of *Tylophora indica* plant ethanol extract

peak shows the presence of toluene, 2-Pentadecanone, 6,10,14-trimethyl-, 5-Amino-2H-1benzofuran-3-one, Benzoic acid, were shows the antioxidant, antibacterial and antifungal.

DISCUSSION

The result of GC-MS is used to confirm the complex presence in *Tylophora indica* extract that the existing

Determination of Antioxidant Activity of *Tylophora indica* (burm. F.) Merr. by DPPH Assay

Conc. µg/ml	H ₂ O extract			Ethanol extract			AA		
	A	%	IC ₅₀	A	%	IC ₅₀	A	%	IC ₅₀
50	0.701	60.73	111.43	0.160	91.04	94.83	0.097	94.57	91.91
100	0.558	68.74		0.153	91.43		0.092	94.85	
150	0.398	77.7		0.142	9		0.087	95.13	
					2.04				
200	0.288	83.87		0.129	92.77		0.084	95.29	
250	0.203	88.63		0.126	92.94		0.079	95.57	
300	0.152	91.48		0.118	93.39		0.075	95.8	

DISCUSSION

As shown in above table, the ethanol extracts demonstrated equivalent and considerable concentration-varying scavenging of free radicals between 91.54% to 93% & 91.04% to 93.39%, for example, in comparison with the standard AA (94.57% to 95.8%). The Aqueous extract from leaves had moderate activity, which ranged from 60.73% -

91.48%. The DPPH assay yielded IC₅₀ values of 94.83 µg/ml for ethanol extraction and 111.4 µg/ml for Aqueous extraction. The IC₅₀ value of the common antioxidant was 127.737 µg/ml. Considerable antioxidant activity is shown by low IC₅₀ values. With the lowest IC₅₀ value at higher doses, the ethanol extract thus demonstrated the highest antioxidant properties of the other leaf extracts.

Determination of Antioxidant Activity of *Tylophora Indica* (burm. F.) Merr. by ABTS assay

Conc. µg/ml	H ₂ O extract			Ethanol extract			AA		
	A	%	IC ₅₀	A	%	IC ₅₀	A	%	IC ₅₀
50	0.465	12.48	334.93	0.423	20.38	256.9	0.297	44.1	127.7
100	0.443	16.62		0.401	24.52		0.242	54.45	
150	0.387	27.16		0.344	35.25		0.183	65.56	
200	0.374	29.61		0.331	37.7		0.142	73.27	
250	0.347	34.69		0.304	42.78		0.085	84	
300	0.339	36.19		0.299	43.72		0.051	90.4	

DISCUSSION

As can be seen from the preceding table, the amount of inhibition rises in tandem with the extract concentration. The antioxidant capability of the extract that neutralizes ABTS⁺ free radicals has been assessed using ascorbic acid as a reference. At doses of 300 µg/mL, all extracts exhibited the maximum antioxidant capacity. The ABTS⁺ radical-scavenging

capacity of the H₂O extracts was substantially lower than that of the EtOH extract. 90.4% of the typical ascorbic acid (AA) and 61.98% of the entire ABTS⁺ radical were removed by the EtOH extract. The IC₅₀ results for the traditional antioxidants were 127.7 µg/ml, whereas the ABTS assay's readings for H₂O or EtOH was 334.3 and even 256.9 µg/ml, respectively.

Determination of Total Antioxidant Capacity (TAC) of *Tylophora Indica*

(*burm. F.*) Merr. by the Phosphomolybdenum Assay

Conc. µg/ml	H ₂ O extract			Ethanol extract			AA		
	A	%	IC ₅₀	A	%	IC ₅₀	A	%	IC ₅₀
50	0.044	11.36	250.8	0.048	18.75	176.53	0.247	84.21	98.96
100	0.054	27.78		0.059	33.9		0.297	86.87	
150	0.062	37.1		0.076	48.68		0.331	88.22	
200	0.065	40		0.101	61.39		0.368	89.40	
250	0.072	45.83		0.116	66.38		0.409	90.46	
300	0.074	47.3		0.13	68.29		0.451	91.35	

DISCUSSION

The IC₅₀ value for the extract of ethanol was 176.5 µg/ml, while H₂O substance was 250.8 µg/ml. The

reference standard, AA, has a value for the IC₅₀ of 98.96 µg/mL. The MeOH extracts had the IC₅₀ value closest to the standard, indicating significant antioxidant activity.

Determination of Antioxidant Activity of *Tylophora Indica* (*burm. F.*) Merr leave extracts by Hydrogen Peroxide Scavenging (H₂O₂) Assay

Conc. µg/ml	H ₂ O extract			Ethanol extract			AA		
	A	%	IC ₅₀	A	%	IC ₅₀	A	%	IC ₅₀
50	0.453	27.87	180.6	0.399	36.46	156	0.231	63.22	112
100	0.359	42.83		0.308	50.96		0.199	68.31	
150	0.338	46.18		0.298	52.55		0.152	75.8	
200	0.305	51.43		0.244	61.15		0.117	81.37	
250	0.268	57.32		0.207	67.04		0.079	87.42	
300	0.219	65.13		0.198	68.47		0.047	92.52	

DISCUSSION

Most solvent extracts of the investigated plant demonstrated vary in concentration activity in scavenging free radicals. High scavenging ability was demonstrated by the ethanol extract (68.47%). At 300 µg/mL, the H₂O isolates' antioxidant capacity was 65.13%. Ethanol, H₂O extracts from plants, and

average AA extracts had respective IC₅₀ values of 156 and 112 µg/ml. Antioxidant activity was higher in the extract, but it was lowest in the ethanol and water extract. With regards to IC₅₀ (µg/ml) and scavenging capacity at 300 µg/ml, the study evaluated the antioxidant capacity of three extracts from *Tylophora indica* (*burm. F.*) leaves in comparison to standard ascorbic acid.



Free-Radical Scavenging Activity at 300 µg/ml and IC₅₀ Values in µg/ml

Samples	Antioxidant Assay							
	DPPH		ABTS		H ₂ O ₂		TAC	
	%I	IC ₅₀	%I	IC ₅₀	%I	IC ₅₀	%I	IC ₅₀
H ₂ O extract	91.48	111.43	36.19	334.93	65.13	250.75	47.3	180.6
Ethanol extract	93.39	94.83	43.72	256.89	68.47	176.53	68.29	156
AA	95.8	91.91	90.4	127.74	92.52	98.96	91.35	112

DISCUSSION

The inorganic substance of the leaves includes phenolic and tannin components, which have antioxidant properties. These bioactive chemicals have a huge range of biological activities, including antiviral and anticancer activity. The DPPH assay showed that solvent extraction had the strongest antioxidant scavenging activity at 300 µg/mL compared to other assays (see figures below).

CONCLUSION

Phytochemical & Pharmacological Evaluation of *Tylophora indica* (burm. F.) Merr. Extract. Throughout the project, we successfully extracted the active phytochemicals from *Tylophora indica* using an efficient ethanolic extraction method.

Phytochemical analyses reveal that *Tylophora indica* is more amount in bioactive compounds, including flavonoids, phenolic compounds, alkaloids, & terpenoids, which contribute to its antioxidant activity. Notably, hydromethanolic leaf extracts demonstrated significant radical scavenging capacities, with inhibition rates of 93.48 % for DPPH, 43.72 % for ABTS, and 68 .47 % for hydroxyl radicals, comparable to standard antioxidants like ascorbic acid. These results submit that *T. indica* holds promise as a ordinary source of antioxidants, potentially beneficial in preventing diseases associated with oxidative stress.

Among three extracts Ethanolic extract gives more yield and DPPH Assay gives more inhibition rates when compared to others.

REFERENCES

1. Pryer KM, Schneider H, Smith AR, Cranfill R, Wolf PG, Hunt JS, Sipes SD. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature*. 2001 Feb 1;409(6820):61822.
2. El-Radhi AS. History of fever. *Clinical Manual of Fever in Children*. 2018:287-97.
3. Fowler MW. Plants, medicines and man. *Journal of the Science of Food and Agriculture*. 2006 Sep;86(12):1797-804.
4. Sofowora A. Research on medicinal plants and traditional medicine in Africa. *The Journal of Alternative and Complementary Medicine*. 1996 Sep 1;2(3):365-72.
5. Mandal SC, Nayak AK, Dhara AK, editors. *Herbal biomolecules in Healthcare applications*. Academic press; 2021 Oct 5.
6. Awuchi CG. Medicinal plants: the medical, food, and nutritional biochemistry and uses. *International Journal of Advanced Academic Research*. 2019 Jan;5(11):220-41.
7. Salgueiro L, Martins AP, Correia H. Raw materials: the importance of quality and safety. A review. *Flavour and Fragrance Journal*. 2010 Sep;25(5):253-71.
8. Mafimisebi TE, Oguntade AE. Preparation and use of plant medicines for farmers' health in Southwest Nigeria: socio-cultural, magico-religious and economic aspects. *Journal of Ethnobiology and Ethnomedicine*. 2010 Dec;6:1-9.
9. Egbaria K, Weiner N. Liposomes as a topical drug delivery system. *Advanced drug delivery reviews*. 1990 Sep 1;5(3):287-300.
10. Lau ET, Steadman KJ, Cichero JA, Nissen LM. Dosage form modification and oral drug delivery in older people. *Advanced drug delivery reviews*. 2018 Oct



- 1;135:75-84.
11. Jasti BR, Abraham W, Ghosh TK. Transdermal and Topical drug delivery systems. In Theory and practice of contemporary pharmaceutics 2021 Feb 25 (pp. 423-454). CRC Press.
 12. Broadhead J, Gibson M. Parenteral dosage forms. InPharmaceutical preformulation and formulation 2016 Apr 19 (pp. 337-359). CRC Press.
 13. Jordan SA, Cunningham DG, Marles RJ. Assessment of herbal medicinal products: challenges, and opportunities to increase the knowledge base for safety assessment. Toxicology and applied pharmacology. 2010 Mar 1;243(2):198-216.
 14. Efferth T, Koch E. Complex interactions between phytochemicals. The multi-target therapeutic concept of phytotherapy. Current drug targets. 2011 Jan 1;12(1):122-32.
 15. Bandaranayake WM. Quality control, screening, toxicity, and regulation of herbal drugs. Modern phytomedicine: turning medicinal plants into drugs. 2006 Sep 20:2557.
 16. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecules. 2016 Apr 29;21(5):559.
 17. Elujoba AA, Odeleye OM, Ogunyemi CM. Traditional medicine development for medical and dental primary health care delivery system in Africa. African journal of traditional, complementary and alternative medicines. 2005;2(1):46-61.
 18. Moquin B, Blackman MR, Mitty E, Flores S. Complementary and alternative medicine (CAM). Geriatric Nursing. 2009 May 1;30(3):196-203.
 19. Pryer KM, Schneider H, Smith AR, Cranfill R, Wolf PG, Hunt JS, Sipes SD. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature. 2001 Feb 1;409(6820):61822.
 20. Nagar PS. Floristic biodiversity of Barda Hills and its surroundings. Scientific Publishers; 2005 Sep 1.
 21. Vivean PR, Kumar V, Manigandan LS, Sasikala M, Parthibhan P. Tylophora indica A Mini Review. Int. J. Front. Sci. Technol. 2014;2:58-66.
 22. Müller-GoymannCC. Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration. European Journal of Pharmaceutics and Biopharmaceutics. 2004 Sep 1;58(2):343-56.
 23. Knaak JB, Leung HW, Stott WT, Busch J, Bilsky J. Toxicology of mono-, di-, and triethanolamine. Reviews of environmental contamination and toxicology. 1997:1-86.
 24. Shilpa A, Agrawal SS, Ray AR. Controlled delivery of drugs from alginate matrix. Journal of Macromolecular Science, Part C: Polymer Reviews. 2003 Jan 6;43(2):187-221.
 25. Gujrati V, Patel N, Rao VN, Nandakumar K, Gouda TS, Shalam MD, Kumar SS. Hepatoprotective activity of alcoholic and aqueous extracts of leaves of Tylophora indica (Linn.) in rats. Indian journal of pharmacology. 2007 Jan 1;39(1):43-7.
 26. Whittig LD, Allardice WR. X - ray diffraction techniques. Methods of Soil Analysis: Part 1 Physical and Mineralogical Methods. 1986 Jan 1;5:331-62.
 27. Maheshwari M, Vijayarengan P. Phytochemical screening and GCMS analysis of Tylophora indica. Int. J. Bot. Stud. 2020;5:255-75.
 28. Ali AH. High-performance liquid chromatography (HPLC): A review. Annals of advances in chemistry. 2022 Jun 20;6(1):010-20.
 29. Erhonyota C, Edo GI, Onoharigho FO. Comparison of poison plate and agar well diffusion method determining the antifungal activity of protein fractions. Acta Ecologica Sinica. 2023 Aug 1;43(4):684-9.
 30. Kristanti D, Herminati A. Characteristics of physical, chemical, and organoleptic properties of inulin-enriched pudding as a complementary food. InIOP Conference Series: Earth and Environmental Science 2019 Mar 1 (Vol. 251, No. 1, p. 012032). IOP Publishing..

HOW TO CITE: M Senthilraja, S Hemanathan, Phytochemical And Pharmacological Evaluation Of Tylophora Indica (Burm. F.) Merr. Extract For Antioxidant Activity, J. Pharm. Sci., 2026, 2 (4), 1532-1550. <https://doi.org/10.5281/zenodo.19503191>

