THERAPEUTIC POTENTIAL OF

Withania somnifera and Celastrus paniculatus

Major project report submitted in partial fulfilment of the requirement for the degree of Masters in Science in Biotechnology

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DECLARATION

I do hereby declare that this dissertation is titled "Therapeutic potential of *Withania somnifera and Celastrus paniculatus*" submitted towards attainment for the award of degree of Masters in Science in biotechnology under the guidance of Dr. Gopal Singh Bisht, Department of biotechnology and bioinformatics, Jaypee university of information and technology, is wholly based on the study and results carried out. Therefore, the declaration made by the student is true and genuine.

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CERTIFICATE

This is to certify that the work which is being presented in the project report titled "Therapeutic potential of *Withania somnifera* and *Celastrus paniculatus*" in partial fulfilment of the requirements for the award of the degree of M.Sc in Biotechnology and submitted to the Department of biotechnology, Jaypee University of Information Technology, Waknaghat is an authentic record of work carried out by " Ritika Goswami (217802)" during the period from June 2022 December 2022 under the supervision of "Dr. Gopal Singh Bisht", Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology, Waknaghat.

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ABSTRACT

Plants and plant products have been utilised as medicines since the start of history. Despite advancements in synthetic medications and antibiotics, plants still occupy a major place in modern as well as traditional system of medicine all over the world. Natural herbal remedies have now become vital for the management and healing of many disorders due to the negative effects of modern medicine and the lower cost of herbal goods. The objective of my study was to understand the therapeutic potential of *Withania somnifera* and *Celastrus paniculatus* by analysing their phytochemical constituents, antimicrobial activity, antioxidant and antiinflammtory activity. The results revealed significant antioxidant and antiinflammatory activity in both selected plants. Methanolic, ACN and hydroethanolic extracts were tested against test organisms. Methanolic and hydroethanolic portions showed antimicrobial activity against the test organisms with zone of inhibition ranging from 5-31mm. The work provides a scientific foundation for the use of plant extracts in the development of new drugs and the treatment of microbial-induced illnesses.

CHAPTER 1: INTRODUCTION

1.1 Medicinal plants: Future source of new drugs

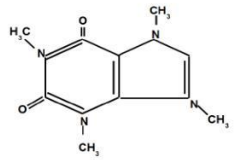
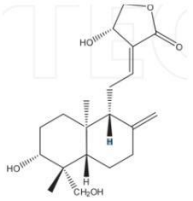
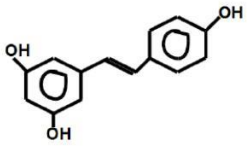
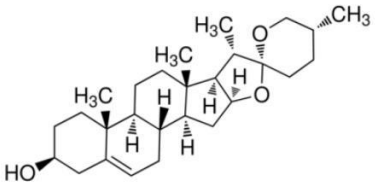
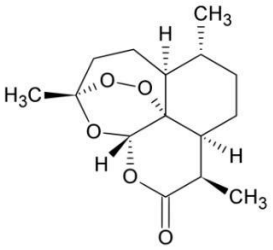
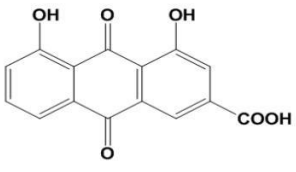
Ayurveda, the traditional herbal medicine system, has a lengthy history and a firm foundation in India. Plants have been used as traditional medicine for thousands of years. Plants have been linked to the advancement of human civilization all throughout the world. Plants, on the other hand, are thought to be rich suppliers of phytochemical compounds, which enable them to have medical benefit. Medicinal plants have the potential to be a source of innovative herbal medications. The pharmacological effects of medicinal plants have been identified as a promising future drug/medicine for the management of health care in the twenty-first century. There has been a renaissance of interest in rediscovering medicinal plants as a source of possible medication candidates in recent years. [1]

1.2 Herbal Medicine

Herbal medicine, also known as phytomedicine, is the use of plants for medicinal and therapeutic purposes in the treatment of diseases and the improvement of human health. Plants have secondary metabolic compounds known as phytochemicals ('phyto' comes from Greek and means 'plant').

These chemicals protect plants against microbiological diseases or pest infestations. Phytochemicals are active compounds that have therapeutic qualities and are used as medicine or drugs [2].

Table 1: Classification of phytochemicals

1.	Alkaloids	 Caffeine	Morphine, caffeine, Berberin, codeine [3]
2.	Glycosides		Amygdalin, gentiopicrin, and rographolide, polygalin, Cinnamyl acetate [4]
3.	Polyphenols (flavonoids, Phenolics, Tannins)	 Resveratrol	Quercetin, reservaratrol kaempferol and quercitrin, caffeic acid, flavones, rutin, naringin, hesperidin and chlorogenic, tannic acid, gallic acid and ellagic acid [5]
4.	Saponins		Diosgenin and hecogenin [6]
5.	Terpenes (Carotenoids, steroids)		Artemisinin, α-carotene, β-carotene, lycopene, lutein and zeaxanthin [7]
6.	Anthraquinones	 Rhein	Rhein, salinos poramide and luteolin [8]

1.3 Development of herbal drugs and its challenges

Plant drug development began with the development of chemistry, isolation, purification, and characterisation of plant active chemicals. Herbal medication is more effective, has less side effects, and is less expensive than allopathic medicine. Herbal medications include herbs, herbal materials, herbal preparations, and herbal products that contain active substances derived from plants or other plant components. Herbal plants and their derivatives have long been known to play important roles in current medicine development [9]. Despite the success of drug development research based on medicinal plants over the last 2-3 decades, future endeavours confront numerous hurdles. The quality of a herbal product is called into question; raw material standardisation emerges as a major issue for the herbal sector [10]. Herbal plants are easily polluted during the growing, processing, and collection processes.

The two most serious issues with herbal medicines are adulteration and heavy metal contamination[11]. As a result, it is critical to increase the quality and quantity of bioactive chemicals in order to produce novel herbal drugs and keep up with other drug development efforts [12]. There are various therapeutic plants available today, and their bioactive substances have been scientifically and clinically tested by researchers.

1.4 OBJECTIVE

The objectives of this study are :

1. To evaluate the different phytochemicals present in plant extracts.
2. To evaluate the antimicrobial, antiinflammatory and antioxidant activity of plant extracts.
3. To understand which plant can be a potential candidate for the development of future drugs.

CHAPTER 2: REVIEW OF LITERATURE

2. INTRODUCTION

Natural products were the source of all drugs in ancient times, and higher plants supplied the majority of the therapeutic agents. In current times, organic compounds and their derivative products are found to contribute for nearly half of all drugs in medicinal use around the globe and 25% of global drug production. [16] According to the WHO, traditional medicine is used by 80 percent of the population in developing countries for basic health care, and 85 percent of traditional medicine depends only on plant extracts. This indicates that plants are used as a resource of pharmaceuticals by about 3.5 to 4 billion individuals throughout the globe. [17]

2.1 TRADITIONAL MEDICINE

Herbology is the practice of using herbs to heal various illnesses and physiological abnormalities in Ayurveda, Siddha, and Unani. In India's AYUSH systems, around 8,000 herbal remedies have been compiled. In the vast majority of cases, traditional medical systems are still widely used. Massive plants have been used in India for many decades to create a variety of medicinal preparations for both external and internal use.

Ayurveda, the traditional Indian system of medicine, is based on the holistic treatment of diseases that primarily rely on natural herbal drugs. Ayurveda is by far the most primitive of all traditional medicines. It is thought to be older than traditional Chinese medicine and is said to source of systematic medicine.[18]

Table 2 : Various medicinal plants showing anti-microbial activity

PLANT NAME	ACTIVITY SHOWN
1. *Juniperus communis*	Antibacterial & antifungal [15]
2. *Urtica dioica*	Antibacterial & anti-fungal [15]
3. *Coleus forskohlii*	Antibacterial & anti-fungal [15]
4. *Pholidota articulate*	Antimicrobial [16]
5. *Dioscorea deltoidea*	Anti-microbial [16]
6. *Pinus roxburghii,*	Antimicrobial, antibacterial & anti-fungal [17]
7. *Pinus wallichiana*	Antimicrobial, antibacterial & anti-fungal[17]
8. *Arnebia benthamii*	Antimicrobial & anti-oxidant [18]
9. *Pyrus pashia*	Anti-microbial, anti-fungal [19]

10. Araucaria cunninghamii	Anti-fungal [20]
11. Biota orientalis	Anti-fungal [21]
12. Cedrus deodara	Anti-fungal [21]
13. Cupressus torulosa	Anti-fungal [21]
14. Prunus cornuta	Anti-microbial [22]
15. Quercus semicarpifolia	Anti-microbial. [22]
16. Taxus wallichiana	Anti-microbial, anti-fungal [23]
17. Hypericum perforatum	Anti-microbial [23]
18. Cymbopogon citrate	Anti-fungal[22]
19. Rumex dentatus	Anti-microbial, anti-fungal [24]
20. Terminalia arjuna	Anti-microbial, anti-fungal[23]

There are many antimicrobial and anti-fungal drugs available in the market these include Penicillin, world's first antibiotic, Valacyclovir and Fluconazole which combat viruses and strains of fungi capable of causing infections[10]. Different anti-microbial substances work by interfering with the formation of cell walls, plasma membrane integrity, nucleic acids, ribosome function, and folate synthesis. ß-lactams like penicillins and cephalosporins, prevent peptidoglycan polymerization, thus preventing the formation of cell walls[11]. Polyenes and imidazoles attack the plasma membrane proteins of fungi. Quinolones prevent DNA replication by attaching to a bacterial combination of DNA and gyrases[11]. Rifampin binds to DNA guided RNA polymerase and prevents the production of new RNA. The ribosome is affected by aminoglycosides, tetracycline, chloramphenicol, erythromycin, and clindamycin. Trimethoprim and sulfonamides disrupt the synthesis of folate needed for DNA replication. To achieve the huge numbers found during an illness or on the smooth surfaces of the body, bacterial cells proliferate regularly. Organisms need to synthesis or take up a wide variety of biomolecules in order to multiply and divide[12]. Antimicrobial drugs obstruct particular processes required for growth and/or division. Despite the fact that bacteriostatic treatments allow the host's natural defences to kill the bacteria, bactericidal agents are more effective. The peptidoglycan layer is the crucial target of anti-cell-wall drugs[13]. Because the stiffness of the bacterial cell wall is destroyed when this layer is lost or damaged, bacteria cannot survive in hypotonic conditions. Three phases of peptididoglycan production take place. Several antimicrobial substances obstruct these initial stages of cell wall production[13]. The enzyme phosphoenolpyruvate converts UTP and N-acetylglucosamine -1-P to UDP-N-

acetylglucosamine[13]. By making a nucleophilic attack on the enzyme, fosfomycins prevent this transfer, with no effect seen on the host. Sterols are present in fungal membranes but not in bacterial membranes[14]. The polyene antibiotics have a hard

hydrophobic centre and a flexible hydrophilic part, and they appear to work by attaching to membrane sterols. The polyene component of polyenes holds the rods packed in a rigid manner. They work with the cells of the fungus to create a membrane-polyene complex that changes the permeability of the membrane, causing the fungus to become internally acidic. The polyene creates pores in the fungal membrane allowing the fungal contents to flow out. Other substances prevent the production of fungal lipid membranes[14]. These medicines—miconazole, ketoconazole, clotrimazole, and fluconazole—belong to a group of substances known as imidazoles. Numerous substances prevent the synthesis of purines and pyrimidines, as well as the interconversion or use of nucleotides. Other substances function as polynucleotide analogues of nucleotides. Yeast species are inhibited by the antifungal chemical flucytosine (5-fluorocytosine), which blocks the thymidylate synthesase, eventually affecting in DNA synthesis[14]. Acyclovir, prevents the thymidine kinase and DNA polymerase of the many herpes viruses after being converted to a triphosphate. Zidovudine (AZT) prevents the reproduction of the human immunodeficiency virus (HIV) by blocking the viral RNA-dependent DNA polymerase[14].

2.2 Selection of plants for the present study

Based on a comprehensive analysis of the literature, the following plants were chosen for the study

1. *Withania somnifera* (Aswagandha)
2. *Celastrus paniculatus* (Malkandi)

2.2.1 Withania somnifera

Withania somnifera (family. Solanaceae) aka Aswagandha is a medicinal plant native to the Indian subcontinent. Its roots are grinded and used in the preparation of various Ayurvedic formulations. It was widely used to treat a variety of biological problems in humans, either alone or in combination with other herbs. It has a range of beneficial chemical properties, including antimicrobial, antistress, antitumor, neuroprotective, cardioprotective, and many more. Furthermore, it was able to reduce reactive oxygen species[ROS] and regulate apoptosis.

1A

1B

1C

Figure 1A: The view of the entire plant of *Withania somnifera.*
Figure 1B: Leaves of *Withania Somnifera.*
Figure 1C: The red berries(fruits) of *Withania somnifera*.

2.2.1.2 Taxonomical classification of *Withania somnifera*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Solanales
Family	Solanaceae
Genus	Withania
Species	*W.somnifera*

Table 3: Scientific classification of *Withania somnifera*

2.2.1.3 Botanical description of *Withania somnifera*

Withania somnifera (family. Solanaceae) aka Aswagandha is a medicinal plant native to the Indian subcontinent. It's a woody shrub that goes by the names "Indian ginseng" or "winter cherry." It is a small shrub or herb grown as an annual plant in colder regions, however it grows as a ground covering perennial in its native habitat. Its leaves grow in a composite manner and have a typical odor. It also consists of small red berries which are the fruits of the plant along with white flowers. Its roots are grinded and used in the preparation of various Ayurvedic formulations.

2.2.1.4 Active Compounds of Withania somnifera

The most abundant active component in Aswagandha is withanolides. withaferin A have been associated with anti-inflammatory [25] and immunosuppressive properties, whereas sitoindosides IX and X are immunostimulatory [26]. Withanolide D has antitumour activity and sitoindosides VII and VIII are antioxidants Other withanolides, including their glycosylated products are reported to have immunomodulatory and other activities [27].

2.2.1.5 Therapeutic properties of Withania somnifera

- Antioxidant Activity

  Antioxidant compounds neutralize the cells from free radicals which are produced as part of the body's regular metabolic function but free radicals above a certain level can harm body cells. Antioxidant capacity refers to the measure of the number of free radicals captured by the antioxidant sample. The brain and nervous system are more susceptible to free radical

damage, since they are made up of lipids and iron. Free radical damage of the nervous system can lead to a number of neurodegenerative diseases [28]. Because traditional Ayurvedic use of WS encompassed numerous ailments related with free radical oxidative damage, it was thought likely that the effects were due to a certain level of antioxidant activity. Sitoindosides VII-X and withaferin A have been tested to reduce the concentration of free radicals using the major free radical scavenging enzymes like SOD, CAT AND GPX levels in mice models [29].

- Antitumor Activity

  Flavonoids has been reported to have anti-cancer properties and intaking food with high flavonoid content reduces the cancer risk. Withaferin A found in WS has been proven to have antitumor effects in mice models [30]. Along with antitumor activity, radiosensitizing effects of withaferin A have also been reported [31].

- Antistress Activity

  Withania somifera has been proven to reduce stress by inducing a state of nonsoecific increased resistance to it [32]. Sitoindosides VII and VIII are the active compounds that have exhibited high anti-stress activity in a number of mice models [33]. WS works in stress induced conditions like swimming and other high endurance activities by building tolerance against it. These results could support the use of *Withania somnifera* in reparing nervous exhaustion caused in humans.

- Anti-inflammatory Activity

  The anti-inflammatory effects of ashwagandha may contribute to its success in a number of rheumatologic disorders. WS has been reported to be a better candidate in reducing inflammations than drugs used for the same purpose (eg, hydrocortisone 15mg/mL). Due to its effective role in reducing inflammations, WS can be used for the treatment of arthritis [34].

- Immunomodulatory Activity

  The usage of WS as a general tonic to boost energy and prevent disease may be related to its immune-boosting properties. Withanolides and sitoindosides IX and X have been reported for their immunomodulatory and nervous system regulation effects. Both compounds have

been proven to statistically significant mobilization and activation of peritoneal macrophages, phagocytosis, and enhanced lysosomal enzyme activity [35].

- Nervous system effects

  Total alkaloid extract of WS roots have exhibited a taming effect and a mild tranquilizer effect on the central nervous system of various animal models like monkeys, rats, mice, cats and dogs. Sitoindosides VII-X and withaferin have been studies for producing these effects [36].

2.2.2 Celastrus paniculatus

Celastrus paniculatus Willd. (CP) is a big, woody, climbing shrub found virtually everywhere in India up to an altitude of 1800 m and is noted for its capacity to increase memory. [37] Ayurveda, has used this plant seed for disease prevention and treatment.

2.2.2.1 Taxonamical classification of *Celastrus paniculatus*

Table 4: Scientific classification of *Celastrus paniculatus*

Kingdom	Plantae
Division	Angiosperms
Class	Tracheophytes
Order	Celastrales
Family	Celastraceae
Genus	Celastrus
Species	C.paniculatus

2.2.2.2 Botanical description of *Celastrus paniculatus*

Celastrus paniculatus (CP) is a climbing shrub with terete branches, the young shoots are pendulous. Flowers are unisexual, flower throughout the year (pendulous panicles) and yellowish green. The fruit of the plant is capsule, glovose, 3 to 6 seeded. The leaves are broadly ovate or obovate and acute. The seeds of the plant are completely enclosed in a red arillus and is brown.

2A

2B

Figure 2A: The view of the leaves of *C.paniculatus*
Figure 2B: Fruits of *C.paniculatus.*

2.2.2.3 Active compounds of *Celastrus paniculatus*

The CP seed aqueous extract included tannins and reducing sugars but no starch. Malkanguniol is one of several sesquiterpene polyalcohols found to be present in the saponified 80% methanolic extract of seed oil. In addition to malkanguniol, four related alcohols were recovered from the extract: polyalcohol A, polyalcohol B, polyalcohol C, and polyalcohol D [38]. The petroleum ether extract of the fruits revealed the presence of steroids/terpenoids, alkaloids, but not flavonoids or saponins. [39] The polyalcohol dulcitol was extracted from the CP flowers. This was the first time it has been reported in the genus Celastrus. Malkangunin, a novel sesquiterpene ester, and three sesquiterpene alkaloids (celapanin, celapanigin, and celapagin) were identified from CP[40].

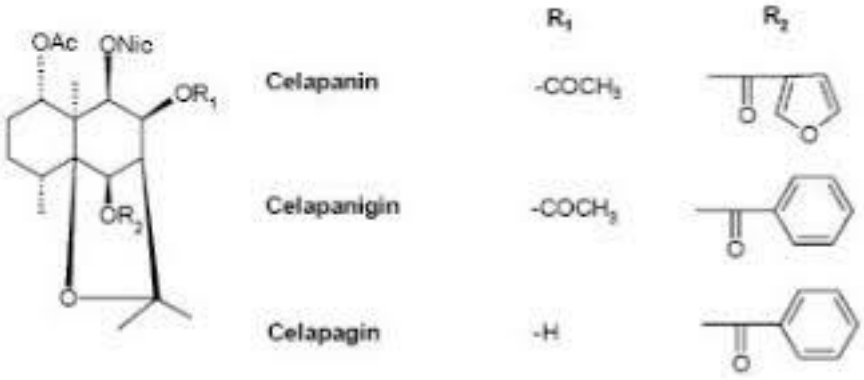


Figure 3: Sesquiterpene alkaloids in *Celastrus paniculatus*

2.2.2.4 Therapeutic properties of *Celastrus paniculatus*

- Analgesic and Anti-inflammatory Activity

  The methanolic extract of the flowers of CP have reported to have analgesic and anti-inflammatory potential.

- Anti-oxidant Activity

  Free radicals can cause harm to body cells due to which free radical scavengers are employed. The methanolic extract of CP has been proven to show an effective free radical scavenging capacity by its ability to scavenge superoxide anion and hydroxyl radicals as well as decrease hydrogen peroxide induced cytotoxity and DNA damage in human fibroblast cells [41].

- Anti-arthritic Activity

  Petroleum ether and alcoholic extracts of CP seeds has been proven to show anti-arthritic effect on Freund's adjuvant arthritis infected albino rats [42].

- Wound healing Activity

  Lupeol, a triterpene compound found in the leaves of CP is found to be effective for wound healing effect in Swiss albino rats. Wound healing activity was found to be greater in lupeol-treated groups than in nitrofurazone-treated groups [43].

- Anti-malarial Activity

  Pristimerin, a quinonoid triterpene found in the root bark of CP has been proven to show anti-malarial activity [44]. Its effective in-vitro against *P.falciparum* though the exact mechanism is still unknown.

- Anti-bacterial Activity

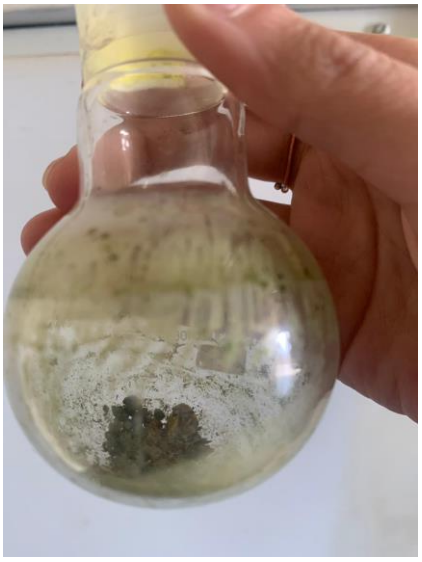
  CP seed oil has shown anti-bacterial activity against a number of gram positive and gram negative bacteria. CP seed oil has shown activity against *Bacillus cereus, Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Salmonella typhosa, Salmonella partyphi A, Pseudomonas aeruginosa, Staphylococcus lutea* [45]. In my study the hydroethanolic extract of leaves of CP has shown anti-bacterial activity against *Escherichia coli* and *Bacillus subtitlis.*

- Anti-fungal Activity

  CP extracts have been studied for its inhibitory activity against six fungal species (*Trichophyton mentagrophytes, Trichophyton rubrum, Trichophyton soudanense, Candida albicans, Torulopsis glabrata, and Candida krusei*) [46].

CHAPTER 3 : MATERIALS AND METHODS

1. Collection of plant samples: All three plant samples were collected from Jaypee University of information technology's herbal garden. They were thoroughly washed under running tap water to remove all soil contaminants. Kept for drying under shade for the next 20 days. After complete drying of the plants, the leaves of all three plant species were grinded to powdered form using a grinder.
2. Extraction of bio-active compounds: Plant extracts were prepared using two major techniques; maceration and Soxhlet extraction.
    - **Maceration**: In this technique, methanol and acetonitrile were used in order of decreasing polarity. 5g of dried sample was soaked in 200Ml of solvents and kept soaked for 36 hours. The solution was filtered using 2um Whatman filter paper and then solvent was evaporated using a rotary evaporator. After complete evaporation of solvent, samples were lyophilised to dryness. Crude extracts were then weighed.
    - **Soxhlet extraction:**
    - Low boiling point solvents (eg: di-chloromethane) are usually used.
    - Weighed out10g of powdered tissue containing and 300ml of methanol and placed in a muslin cloth (thimble).
    - Refluxed the sample for 8hr at 40●c using a condensor.
    - Allowed the sample to cool to room temp and filter using 40µm filter paper.
    - Collected liquid is evaporated using a rotary evaporator and then lyophilized to dryness. Crude extract was then weighed.
    - **Rotary Evaporator :** Works by reducing the volume of a solvent at reduced temperature and reduced pressure is known as rotary evaporation. This promotes the removal of excess solvent from volatile samples. The heat bath, condenser, rotor, and solvent trap are the components of most rotary evaporators. An aspirator or vacuum pump, a bump trap and RBF containing the concentrated sample, must also be attached. The temperature should be set close to the boiling point of the solvent.
    - **Lyophilizer :** A lyophilizer is a device that removes aqueous contents from easily perishable materials with the goal of extending their shelf life.



4A 4B

Figure 4A: Crude extract obtained after solvent evaporation

Figure 4B: Crude extract after lyophilisation

3.2 Characterization of plant extract

3.2.1 Qualitative screening of phytochemicals

The methanolic extracts of all three samples was used for the detection of various secondary metabolites.

- **Test for flavanoids**

1. Add few drops of NaOH to 1mL of extract
2. Appearance of yellow colour
3. Add few drops of dilute HCL.
4. Solution will turn colourless as indicator of presence of flavanoids.

- **Test for saponins**

1. Add few drops of 1% lead acetate solution to 1mL of extract.
2. Appearance of white precipates indicates presence of saponins.

- **Test for sterols**

1. Add 2mL of conc H2SO4 to 1mL of extract
2. Formation of red precipitate indicates presence of sterols.

- **Test for tannins**

1. Add 1mL of 3% FeCl3 solution to 1mL of extract.
2. Brownish green colour indicates presence of tannins.

- **Test for phenols**

1. To 2mL of extract, add 2mL of distilled H2o and few drops of 10% FeCl3 solution.
2. Brisk red colour indicates presence of phenols.

3.2.2 Quantitative screening of phytochemicals

a) Estimation of Total Phenolic Content [TPC]

Reagents:

Reagent A: 48 millilitres of 2% sodium carbonate in 0.10 N sodium hydroxide + 1ml 0.5% CuSO4.5H20 in sodium potassium tartrate.

Reagent B: Diluted Folin Reagent (1:1)

Methodology:

Prepare Reagent A by adding 48ml of Na2CO3 in 0.1N NaOH and 1 ml of sodium potassium tartrate in 1 ml of copper sulphate. Reagent B was prepared by taking 1:1 ratio of Folin

reagent in distilled water. 1 millilitre of test sample and 4 millilitres of Reagent A should be mixed together, then left to stand at room temperature for 10 minutes. Add 0.5 millilitre of Reagent B to the mixture. After then, set it aside in the dark for 30 minutes. In a microplate reader, measure the absorbance at 750nm. [47]

b) Estimation of Total Flavonoid Content [TFC]

To make a 10% solution of Quercetin, dissolve 1g of Quercetin in 100 mL methanol. Prepare dilutions of different concentrations (0.1, 0.5, 1.0, 2.5, and 5mg/ml) in methanol from standard quercetin solution to generate a standard gallic acid curve. Allow to stand for 6 minutes after mixing 100 l of each quercetin dilution with 500 ul of distilled water and 100 l of 5% sodium nitrate. After that, add 150 ul of a 10% aluminium chloride solution and let it sit for 5 minutes. After that, gradually add 200 ul of a 1M sodium hydroxide solution. Using a microplate reader, measure the absorbance at 510 nm. Calculate flavonoid content as quercetin equivalents (mgQE/g). [48]

3.3 Biological Activity of plant extract

3.3.1 Evaluation of Antioxidant activity of plant extract.

ABTS assay:

To produce ABTS free radical solution, thoroughly mix ABTS (7 Millimolar) and potassium persulfate (2.45 Millimolar) solutions and incubate 24 hrs in the dark. At 745nm, add methanol to 0.7 to adjust the absorption of this mixture. Incubate for 6 minutes after mixing 300L extract working dilutions with 3.0mL ABTS solutions. Finally, using a microplate reader determine the absorbance at 745nm. Positive control was Gallic acid. The scavenging potential of ABTS in percent was calculated by using the following formula:

$$= \frac{\text{control absorbance- sample absorbance}}{\text{Control absorbance}} \text{ X } 100$$

DPPH scavenging assay:

Make a 0.002% DPPH solution in methanol and measure the absorbance at 515 nm. Mix 50 ul plant extract (1 mg/mL methanol) with 3 ml DPPH solution and set down for 15 minutes in the dark. At 515 nm, record the absorbance once more. Using the formula below, calculate the percentage inhibition of DPPH by plant extracts.

% inhibition = A1-A2/ A1 X 100

Where,

A1= absorbance of pure DPPH

A2= absorbance of sample after DPPH reaction.

3.3.2 Evaluation of Anti-inflammatory activity of plant extract.

Protein Denaturation Assay

2ml of plant extract was taken which was made into varying concentrations (50mg/ml, 125ug/ml, 200ug/ml, 250ug/ml, 500ug/ml). To this 0.2ml of egg albumin was added and 2.8ml of PBS (pH 6.5). The mixture was incubated at 37 degree for 15 mins and then heated at 70 degree for 5 mins. After cooling absorbance was taken at 660nm. Final concentrations used were 125, 200,250 and 500ug/ml. Distilled water served as the control.

% inhibition = 100 x A1/A2 -1

A1= Absorbance of sample

A2= Absorbance of control

3.3.3 Evaluation of Anti-microbial activity of plant extract.

Anti-microbial Susceptibility Test (AST)

Drugs and chemicals used:

- Drugs: Cefotaxime (for bacteria)
- Chemicals: LB Agar, LB broth, distilled water, McFarland solution (BaCl2 and H2SO4)
- Micro-organisms: *Bacillus subtilis* (gram +ve) and *Escherichia coli* (gram -ve)

- **Screening for antimicrobial activity** : Well-diffusion assay
- Bacterial strains were procured from Jaypee University lab.
- Single colonies were picked from culture plates and inoculated into LB broth, which was incubated for 24 hours at 37 degrees. After overnight growth, the turbidity of the cultures were compared to 0.5 Mc Farland standard.
- McFarland standards are used as a reference which represents the volume in (1.5×10^8 CFU/mL)

- 0.5 McFarland Solution: Mix 0.05mL of 1% BaCl2 and 9.95mL of 1% H2SO4

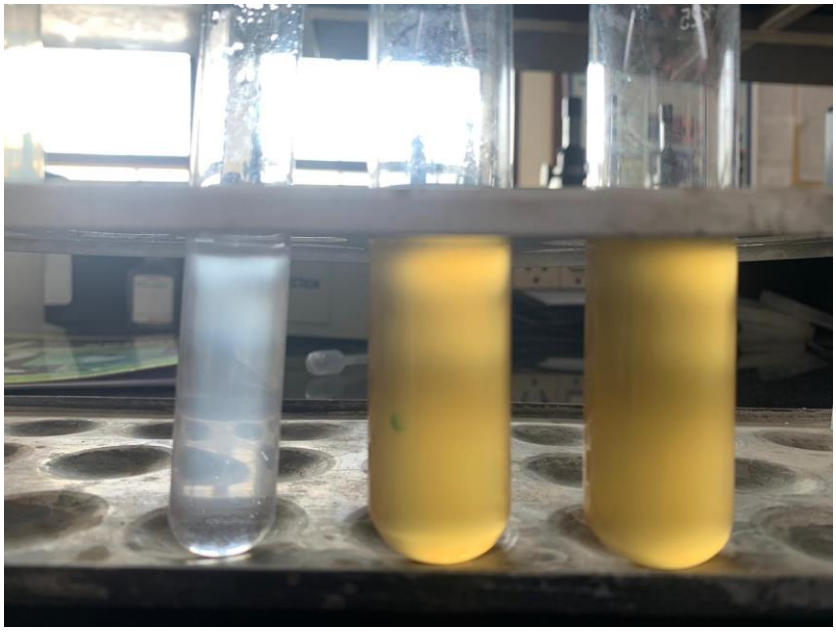


Figure 5: Comparison of bacterial turbidity with 0.5 McFarland standard (represents 1.5 x10^8 CFU/ml)

- Well-diffusion assay was performed for screening, samples with concentrations 5mg/mL, 10mg/mL and 15mg/mL were used.
- A bacterial suspension was prepared with 1.5 x 10^5 CFU/ml by serially diluting the bacterial inoculum.
- 100uL of this bacterial suspension was then spread evenly on LB agar plates.
- Wells were punched and loaded with 100uL of the plant crudes (different concentrations) dissolved in distilled water (sample).
- Positive control used was cefotaxime discs (10mcg/ml)
- The plates were incubated at 37 degrees for 24 hours.
- After incubation, the zones of inhibitions were calculated and checked with the reference ie antibiotic disks. (TABLE 2)
- Activity index for each extract was calculated. (TABLE 3)

CHAPTER 4: RESULTS AND DISCUSSION

4.1 PERCENTAGE YIELD OF PLANT EXTRACT

WEIGHT OF CRUDE EXTRACTS (TABLE 5) :

Plant name	Methanol as solvent	Acetonitrile as solvent
• *W.somnifera*	146mg	33mg
• *M.struthiopteris*	131mg	16mg

WEIGHT OF CRUDE EXTRACTS (TABLE 5)

Plant name	Ethanol as solvent
C.paniculatus	155mg

Dry weight of the powdered plant was 20gm and after solvent evaporation, the crude extract weighed *W.somnifera*: 146mg (methanol), 33mg (ACN)

M.struthiopteris 131mg (methanol), 16mg (ACN)

C.paniculatus. 155mg (hydroethanolic)

Percentage yield was calculated using the following equation:

$$\frac{\text{Weight of extract after evaporating solvent}}{\text{Dry weight of the sample}} \times 100$$

Obtained yield of W.somnifera was 7.3%, M.struthiopteris 6.55% and C.paniculatus 7.75%.

4.2 RESULT OF QUALITATIVE SCREENING OF PHYTOCHEMICALS

Results for qualitative phytochemical analysis tests : (Table 6)

PLANT NAME	FLAVANOIDS	SAPONINS	STEROLS	TANNINS	PHENOLS
Withania somnifera	-ve	+ve	-ve	-ve	-ve
Mattenceia strupthioresis	+ve	+ve	-ve	+ve	+ve
C.paniculatus	+ve	-ve	-ve	+ve	+ve

+ve- shows the presence of plant secondary metabolite

-ve- shows the absence of plant secondary metabolite

4.3 RESULT OF QUANTITATIVE SCREENING O F PHYTOCHEMICALS

Table 7. Result of quantitative screening of phytochemicals

Plants	**Total phenolic content (TPC)** (mg GAE/g)	**Total flavonoid content (TFC)** (mgQE/g)
W.somnifera	308	89
C.paniculatus	32.62	12.53

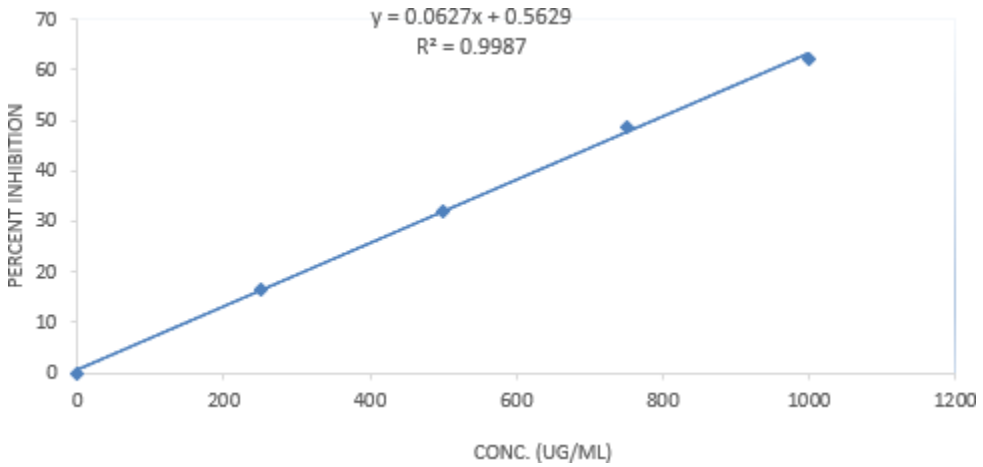
4.4 RESULT OF ANTI0XIDANT ACTIVITY

For DPPH assay : Table 8

Concentration (ug/ml)	**% Inhibition For *W.somnifera***	**% Inhibition For *C.paniculatus***
0	0	0
250	16.46	15.11
500	32.03	23.65
750	48.80	33.83

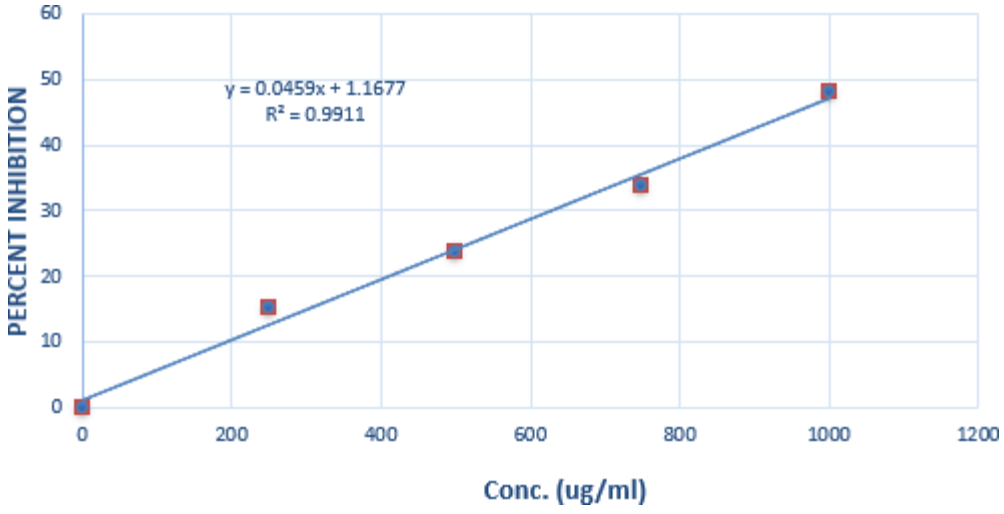
1000	62.15	48.05

Graph of percentage inhibition vs conc of W.somnifera in DPPH assay



IC50 Value was calculated by using this equation: y = 0.0627x+ 0.5629

IC50 value for *W.somnifera* was: 0.788mg in DPPH assay



Graph of percentage inhibition vs conc of C.paniculatus in DPPH assay

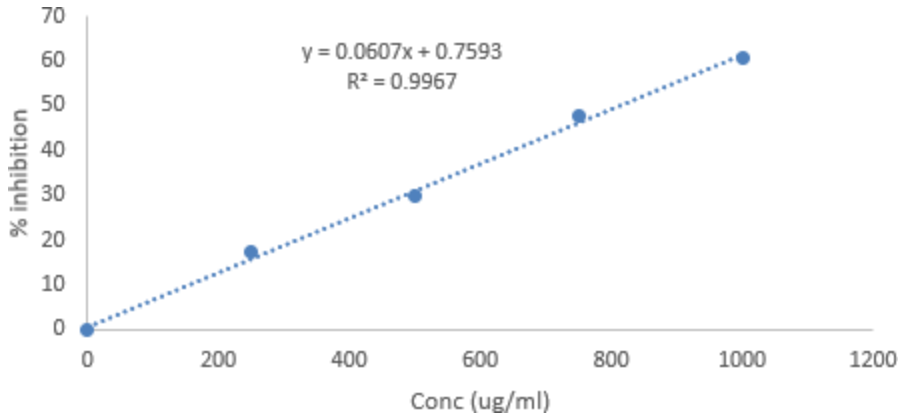
IC50 Value was calculated by using this equation: y = 0.0459x +

1.1677

IC50 value for C.paniculatus was: 1.063mg in DPPH assay

ABTS ASSAY: Table 9

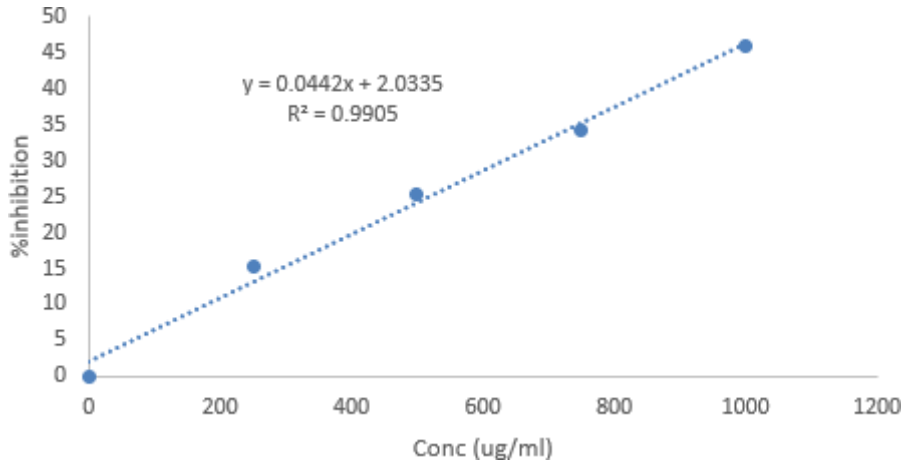
Concentration (ug/ml)	**% Inhibition For *W.somnifera***	**% Inhibition For *C.paniculatus***
0	0	0
250	17.43	15.37
500	29.66	25.22
750	47.77	34.23
1000	60.74	45.81



Graph of percentage inhibition vs. conc. of *W.somnifera* in ABTS assay.

IC50 Value was calculated by using this equation: = 0.0607x + 0.7593

IC50 value for *W.somnifera* was: 0.811mg



Graph of percentage inhibition vs. conc. of *C.paniculatus* in ABTS

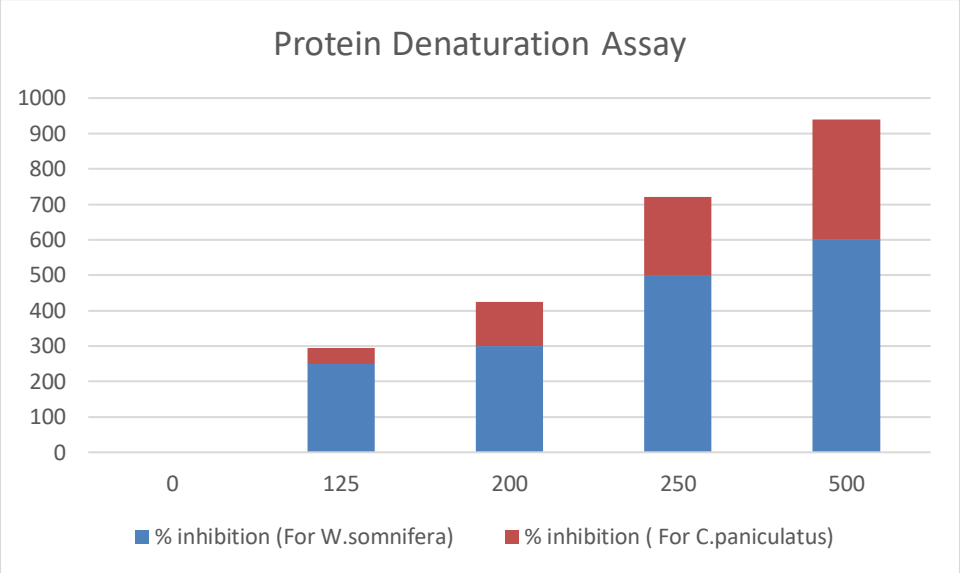
IC50 Value was calculated by using this equation: y = 0.0442x + 2.0335

IC50 value for *C.paniculatus* was: 1.085mg

4.4 RESULT OF ANTI-INFLAMMATORY ACTIVITY

Protein Denaturation Assay (Table 10)

Concentration (ug/ml)	% inhibition (For W.somnifera)	% inhibition (For C.paniculatus)
125	250	45
200	300	125
250	500	220
500	600	340



Graph showing the comparison between W.somnifera and C.paniculatus in protein denaturation assay

4.5 RESULT OF ANTIMICROBIAL ACTIVITY

- Antimicrobial activity : Antimicrobial activity of the three plant extracts were tested against selected micro-organisms. In the lab, total 5 extracts were tested for their bio-activity. Three extracts showed significant anti-microbial potential against the tested micro-organisms. But, the other two extracts showed no activity against any of the selected micro-organisms at the tested concentrations(both extracts of *Matteuccia struthiopteris* showed no bio-activity). Bacillus subtilis was the more susceptible organism as it showed greater zones of inhibition. Maximum anti-microbial activities were recorded for *W.somnifera* (methanolic extracts) against both *E.coli* and *B.subtilis*.
- Activity Index : Activity indexes were calculated for each concentration and for each plant. Since only *W.somnifera* showed zone of inhibition only this plant is used for calculation of activity index. Highest activity index (1.64) was seen at 15mg/ml concentration of *W.somnifera* crude ext against *E.coli*. Hence, 15mg/ml

(of *W.somnifera*) is the MIC at which no growth of the test micro-organism is observed.

ACTIVITY INDEX = ZONE OF INHIBITION OF SAMPLE

ZONE OF INHIBITION OF REFERENCE

Figure 6 : Zone of inhibition (E.coli).

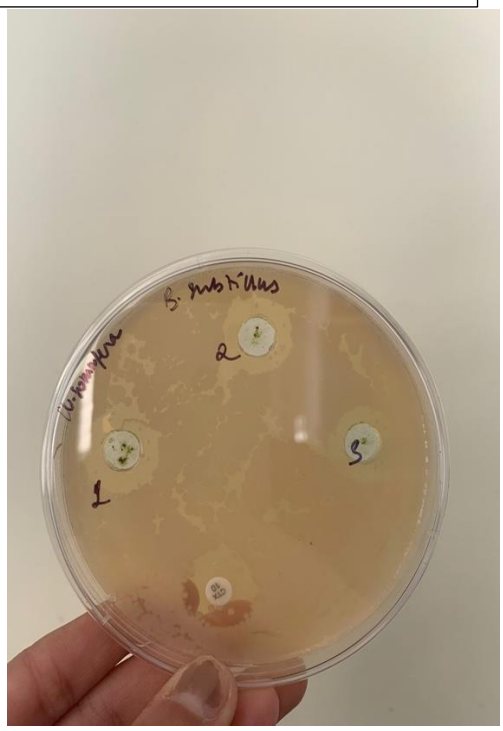


Figure 7: Zone of inhibition (B.subtilis)

For *W.somnifera* : Table 11

Concentration of plant ext	**Zone of inhibition for *E.coli***	**Zone of inhibition for *B.subtilis***
5mg/ml	12mm	31mm

10mg/ml	10mm	30mm
15mg/ml	23mm	27mm
CTX	14mm	30mm

Activity index calculated from the above table (TABLE 12)

Concentration of plant ext	***E.coli***	***B.subtilis***
5mg/ml	0.85	1.03
10mg/ml	0.71	1.0
15mg/ml	1.64	0.9

For *C. paniculatus* : Table 13

Concentration of plant ext	**Zone of inhibition for *E.coli***	**Zone of inhibition for *B.subtilis***
5mg/ml	5mm	3mm
10mg/ml	12mm	10mm

15mg/ml	15mm	12mm
CTX	15mm	30mm

DISCUSSION

- As far as bioactivity is concerned, *W.somnifera* proved to be a better candidate than *C.paniculatus* as it showed greater therapeutic potential in terms of all the activities checked for.
- *W.somnifera*'s activity was more potent against gram positive *B.subtilis* than *E.coli*.

CHAPTER 5: CONCLUSION

The purpose of the present study was to evaluate anti-microbial activity of selected medicinal plants. The present study endeavors to identify and develop new a therapeutic agent for anti-microbial resistance. According to the WHO, almost 75% of the world's population has incorporated the use of medicinal products in their life as an alternate of allopathy drugs. Plant based medicines can serve as better alternative therapeutic agents for the people who live in places where conventional drugs cannot be supplied. However, the scientific evidence of their efficacy is limited therefore it is important to introduce a scientific validation for the medicinal effect of plants used in traditional medicine.

In present study, it has been found that selected plants have strong anti-microbial and anti-oxidant activity and anti-inflammatory activity. An extensive review of literature had revealed that many plants used traditionally as anti-microbial agents also have strong inhibition of protein denaturation and anti-oxidant activities. Thus, the selected plants can be used as potential candidates for rising anti-microbial resistance and development of newer drugs.

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