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Phytochemical analysis of medicinal plants used in the treatment of tuberculosis

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Abstract : Tuberculosis is a pulmonary disease that is caused by Mycobacterium tuberculosis. In the treatment forefront, the drugs rifampicin, isoniazid, pyrazinamide is used and prolonged usage of these drugs results in liver toxicity. This study focuses in depth on the phytochemicals present in medicinal plants Adathoda vasica, Cassia alata, Azadirachta indica, Emblica officinalis and Piper longum and the ability of these bioactive compounds to act as anti-tuberculosis agents**.**

Keywords: Tuberculosis, Adathoda vasica, Cassia alata, Azadirachta indica, Emblica officinalis, Piper longum

1. INTRODUCTION

Traditional Indian medical systems such as Ayurveda and Siddha explain in detail about herbal mixtures that can be used to treat several diseases [1]. Plants produce and store a wide variety of secondary metabolites. The isolation, characterization and determination of these bioactive compounds in medicinal plants play a vital role in formulating drugs[2]. Natural compounds exhibit characteristics such as chemical diversity, macromolecular specificity and less toxicity thus making them favourable leads in the discovery of novel drugs [3]. The synergistic effect of the plant extracts can be used in the treatment of several diseases. Tuberculosis is a highly infectious disease caused by Mycobacterium tuberculosis. In the treatment forefront, the sources are drugs such as rifampicin (RIF), isoniazid (INH), pyrazinamide (PZA) and ethambutol (ETB) [4]. But they cause liver toxicity upon prolonged usage [5]. There is an immediate need for a robust system to improve the effectiveness of drugs.

2. EXPERIMENTAL METHODS

Plant Material Collection: The medicinal plants Adathoda vasica, Cassia alata, Emblica officinalis, Azadirachta indica and Piper longum were collected from different parts of Chennai, Tamil Nadu. The selection was made from previous literature of plants showing anti tuberculosis activity in alternate medicine (Ayurveda). The following table shows the active compound in each plant.

Table 1: List of plants, their active compounds and action against tuberculosis

Plant	Part used	Active compound	Anti Tuberculosis activity	Reference
Adathoda vasica	Leaf	Vasicine (alkaloid)	Antimycobacterial activity against MDR TB (DKU- 156)	[6]
Cassia alata	Whole plant	Kempferol (flavonoid)	Antimycobacterial activity against MDR TB (DKU- 156)	[7]
Azadirachta indica	Leaf	Azadirachtin (steroid)	Induces hepatoprotective activity against anti tuberculosis drugs	[8]
Emblica officinalis	Fruit	Ellagic acid (phenol)	Induces hepatoprotective activity against anti tuberculosis drugs	[9]
Piper longum	Fruit	Piperine (alkaloid)	Effective against Multi Drug Resistant TB in herbal medicine	[10]

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Sample Preparation: The plants were washed well, shade dried, powdered and stored.

Preparation of Extract: The plant samples were extracted in various solvents such was distilled water, ethanol, methanol, acetone, hexane, ethyl acetate and chloroform. 10 g of dried powder was added to 150 ml of the solvents. Extraction was done using Soxhlet apparatus for a time period of 5 hours. It was then stored in bottles for further analysis.

Qualitative Phytochemical Screening: Tests were done to check the presence or absence of all the phytochemicals using standard protocols [11].

Quantitative Phytochemical Screening: Tests were done to check the amount of the bioactive compounds present in each plant from the aqueous extract using standard protocols [11].

3. RESULTS AND DISCUSSION

Qualitative phytochemical screening:

The results obtained from the phytochemical tests done were tabulated.

Table 2: Phytochemical analysis of Adathoda vasica in different solvents

Test	Distilled water	Ethanol	Methanol	Acetone	Hexane	Ethyl acetate	Chloroform
Tannins	+	+	+	+	-	-	-
Saponins	+	+	+	-	-	+	-
Alkaloids	+	+	+	-	-	-	-
Flavonoids	+	-	-	-	-	-	+
Glycosides	+	+	+	-	-	-	-
Quinones	+	+	+	-	+	-	-
Phenols	+	+	+	+	-	-	-
Terpenoids	+	+	+	-	+	-	-
Cardiac glycosides	+	+	+	-	-	-	-
Ninhydrin	-	-	-	-	-	-	-
Coumarin	+	-	-	-	-	-	+
Steroids	+	+	+	-	+	-	-
Phlobatannins	+	-	-	-	-	-	-
Carbohydrates	+	-	-	+	-	-	+

The results obtained for A. vasica using different solvents were as follows. Phytochemicals such as tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, terpenoids, cardiac glycosides, coumarin, steroids, phlobatannins and carbohydrates showed positive results in distilled water. Ethanol and methanol showed positive results for tannins, saponins, alkaloids, glycosides, quinones, phenols, terpenoids, cardiac glycosides and steroids. Acetone, hexane, ethyl acetate and chloroform showed least positive results compared to the other solvent extracts.

Table 3: Phytochemical analysis of Cassia alata in different solvents

Test	Distilled water	Ethanol	Methanol	Acetone	Hexane	Ethyl acetate	Chloroform
Tannins	+	+	+	+	-	+	-
Saponins	+	+	+	+	-	-	-
Alkaloids	+	+	+	-	-	-	-
Flavonoids	+	+	+	-	-	+	+
Glycosides	+	+	+	+	-	-	+
Quinones	+	-	-	-	-	-	+

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Phenols	+	+	+	+	+	+	-
Terpenoids	+	+	+	-	-	-	+
Cardiac glycosides	+	+	+	+	-	-	-
Ninhydrin	+	-	+	+	+	-	+
Coumarin	+	+	+	-	-	-	+
Steroids	+	+	+	-	-	-	+
Phlobatannins	-	+	+	-	-	-	+
Carbohydrates	+	+	+	+	-	+	+

The results obtained for C. alata using different solvents were as follows. Phytochemicals such as tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, terpenoids, cardiac glycosides, ninhydrin, coumarin, steroids and carbohydrates showed positive results in distilled water. Ethanol and methanol showed positive results for tannins, saponins, alkaloids, flavonoids, glycosides, phenols, terpenoids, cardiac glycosides, coumarin, steroids and carbohydrates. Acetone, hexane and chloroform showed lesser number of positive results. Ethyl acetate showed the least number of positive results compared to the other solvents.

Table 4 : Phytochemical analysis of Azadirachta indica in different solvents

Test	Distilled water	Ethanol	Methanol	Acetone	Hexane	Ethyl acetate	Chloroform
Tannins	+	-	+	+	-	+	-
Saponins	+	-	-	-	-	+	-
Alkaloids	+	+	+	-	-	+	-
Flavonoids	+	+	-	-	-	+	+
Glycosides	+	+	+	+	-	+	-
Quinones	+	-	+	+	+	+	+
Phenols	+	+	+	+	-	+	-
Terpenoids	+	+	+	+	-	+	+
Cardiac glycosides	+	+	+	+	+	+	-
Ninhydrin	+	-	-	-	-	+	-
Coumarin	+	+	-	+	-	+	+
Steroids	+	+	+	+	-	+	+
Phlobatannins	-	+	+	+	-	+	-
Carbohydrates	+	+	+	+	-	+	+

The results obtained for A. indica using different solvents were as follows. Phytochemicals such as tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, terpenoids, cardiac glycosides, ninhydrin, coumarin, steroids and carbohydrates showed positive results in distilled water. Ethanol showed positive results for alkaloids, flavonoids, glycosides, phenols, terpenoids, cardiac glycosides, coumarins, steroids , phlobatannins and carbohydrates. Methanol showed positive results for tannins, alkaloids, glycosides, quinones, phenols, terpenoids, cardiac glycosides, steroids, phlobatannins and carbohydrates. Acetone, hexane and chloroform showed lesser number of positive results. Ethyl acetate showed all positive results compared to all other solvent extracts.

Table 5: Phytochemical analysis of Emblica officinalis in different solvents

Test	Distilled water	Ethanol	Methanol	Acetone	Hexane	Ethyl acetate	Chloroform
Tannins	+	+	+	+	-	+	-
Saponins	+	+	+	+	-	+	-
Alkaloids	+	-	+	-	-	-	-
Flavonoids	+	-	+	+	-	-	+
Glycosides	+	+	+	+	-	+	-
Quinones	+	+	+	+	-	-	+
Phenols	+	+	+	+	-	+	-

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Terpenoids	+	+	+	+	+	-	+
Cardiac glycosides	+	+	+	+	-	+	+
Ninhydrin	+	-	+	-	-	-	-
Coumarin	+	+	+	+	-	-	+
Steroids	+	-	+	+	-	+	+
Phlobatannins	-	-	+	+	-	-	+
Carbohydrates	+	+	+	+	+	+	+

The results obtained for E. officinalis using different solvents were as follows. Phytochemicals such as tannins, saponins, alkaloids, flavonoids, glycosides, quinones, phenols, terpenoids, cardiac glycosides, coumarin, steroids and carbohydrates showed positive results in distilled water. Ethanol showed positive results for tannins, saponins, glycosides, quinones, phenols, terpenoids, cardiac glycosides, ninhydrin, coumarin and carbohydrates. Methanol showed all positive results. Acetone showed positive results for all except alkaloids and ninhydrin. Hexane, ethyl acetate and chloroform showed least number of positive results.

Table 6: Phytochemical analysis of Piper longum in different solvents

Test	Distilled water	Ethanol	Methanol	Acetone	Hexane	Ethyl acetate	Chloroform
Tannins	+	-	-	-	-	-	-
Saponins	-	-	+	+	-	-	+
Alkaloids	-	+	+	+	-	-	+
Flavonoids	+	+	+	+	+	-	+
Glycosides	+	-	-	-	-	-	-
Quinones	+	-	+	-	-	-	+
Phenols	+	+	+	-	-	-	-
Terpenoids	-	+	-	+	+	+	+
Cardiac glycosides	-	+	+	+	+	+	+
Ninhydrin	-	-	-	-	-	-	-
Coumarin	+	+	+	-	-	+	+
Steroids	-	-	-	-	-	+	+
Phlobatannins	-	-	-	-	-	-	-
Carbohydrates	+	+	+	+	+	-	+

The results obtained for P. longum using different solvents were as follows. Phytochemicals such as tannins, flavonoids, glycosides, quinones, phenols, coumarin and carbohydrates showed positive results in distilled water. Ethanol, methanol, acetone, hexane and chloroform showed a smaller number of positive results. Ethyl acetate showed the least number of positive results.

Quantitative Phytochemical screening in aqueous extract:

Plant	Tannins (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)	Flavonoids (mg/g)	Phenols (mg/g)
Adathoda vasica	0.27±0.01	0.057±0.002	0.085±0.003	0.23±0.002	0.23±0.01
Cassia alata	0.2±0.05	0.015±0.001	0.334±0.002	0.435±0.014	0.241±0.01
Azadirachta indica	0.46±0.02	0.356±0.042	0.52±0.08	0.245±0.004	0.25±0.02
Emblica officinalis	0.43±0.001	0.040±0.001	1.5±0.08	0.191±0.002	0.13±0.01
Piper longum	0.55±0.05	0.109±0.001	0.105±0.011	0.365±0.017	0.02±0.01

The results showed the amount of bioactive compound present in each plant. Although qualitative phytochemical tests showed the absence of saponins and alkaloids in P.longum , quantitative tests indicate that these compounds are present in concentrations of 0.109±0.001 and 0.105±0.011 mg/g respectively. It could be because quantitative tests performed by spectrophotometry are more sensitive than qualitative phytochemical tests with specific reagents. The other results were found to be in accordance with the qualitative tests. The highest quantity of tannins was found in P. longum

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0.55±0.05 and the least was present in C. alata 0.2±0.05. The highest amount of saponin was found in A. indica 0.356±0.042 and the least amount was seen in C. alata 0.015±0.001. Alkaloids were found to be the highest in E. officinalis 1.5±0.08 and lowest in A. vasica 0.085±0.003. Flavonoids were found to be the most abundant in C. alata 0.435±0.014 and least in E. officinalis 0.191±0.002. Phenols were found to be maximum in A. indica 0.25±0.02 and minimum in P.longum 0.02±0.01.

The plants Adathodha vasica, Cassia alata, Azadirachta indica, Emblica officinalis and Piper longum have innumerable medical benefits. The phytochemical results give as an insight on the bioactive compounds that are present in the plant samples. Either a single compound or a combination of bioactive compounds in plants act against diseases. Using this knowledge, it's possible to come with up combinations that show a synergistic effect against tuberculosis.

In a paper by Nithyatharini [12], the methanolic extract of leaves of A. vasica showed the presence of high number of phytocompounds in comparison with other solvents like ethanol, petroleum ether, chloroform and distilled water. In a study by P Raji [13], the presence of flavonoids, terpenoids and alkaloids in C. alata were seen better in chloroform extract compared to distilled water. From this she inferred that the active molecule could be of polar nature as they were extracted in the solvents of corresponding polarity. Leaf extract of A. indica showed excellent amount of phenolic content 241.642±0.000797874 mg/g in a paper by Ankita Sharma[14]. Amongst the five plants were, phenols were found to be maximum in A. indica. In a study by Dasrao Patil [15], phytochemical investigation of ethanolic extract of Emblica officinalis showed the absence of carbohydrates, tannins, glycosides, flavonoids, terpenoids and saponins. In a study by Gayathri Nahak [16] using different extracts of P. longum, the phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in ethanolic extracts better than methanolic and aqueous extracts.

In the current study, aqueous solutions of the plant samples have shown positives for the major bioactive compounds and hence would be the most ideal as it is readily available, cheap and nontoxic. But the extract that shows maximum anti tuberculosis activity would be known only after in vitro and in vivo studies.

The pressing need for development of drugs using phytochemicals has resulted in research of techniques to tweak existing natural compounds. Most of the phytochemicals are water-soluble, but demonstrate a low absorption [17]. This is due to their inability to cross lipid membranes resulting in reduced efficacy [18].

4. FUTURE PERSPECTIVE

The plant extracts will be tested both individually and in combination against Mycobacterium tuberculosis strains such as standard H37RV and other isolates. Based on the results, studies will be done up to the molecular level to isolate specific compounds that show maximum antagonistic effect.

5. CONCLUSION

Plants possess bioactive compounds that show antibacterial, antifungal, antiviral, anti-inflammatory, hepatoprotective, antioxidant, anticancer, anti tuberculosis etc activities. Human beings have benefited from these compounds in traditional medicine such as Ayurveda, Siddha and Unani. Although there are a number of natural compounds with therapeutic potential their potency is constrained by poor bioavailability, low absorption, fast metabolism, and rapid systemic elimination. Enhancing the use of herbal medicines to re-discover their full potential in pharmaceutical formulation is a great way to combat infectious diseases.

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