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To Identify the Presence of Phytochemicals in Medicinal Plants Such as Clitoria Ternatea and Pterocarpus Santalinus Using Different Solvents Followed by Anti-Microbial Activity Against E. Coli

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Abstract

There are abundant therapeutic plants out there in the vegetation providing numerous health benefits and waiting to be discovered more about them. There are diversions of plants that provide medicinal aid to various ailments and are differentiated based on the various plant families they belong to. In this study, we focus on the two species that belong to the same family. i.e., Fabaceae which are, *Clitoria ternatea* and *Pterocarpus santalinus*. Fabaceae plant family is believed to consist of humungous health benefits including antimicrobial activities, helping in wound healing, being anti-cancer. The presence of a wide variety of secondary bioactive metabolites, including Alkaloids, Flavonoids, Coumarins, Terpenoids, Sterols, and Phenolic acids which have been frequently reported to be present in various plant species of this Fabaceae family, may explain the variety of medicinal effects of these plants: Since ancient times, the Fabaceae plant family has been employed as a source of medicinal plants due to its extensive distribution. This extensive study has been done to observe the presence of phytochemicals of medicinal plants such as *Clitoria ternatea* and *Pterocarpus santalinus* using different solvents followed by anti-microbial activity against *E. coli*, and the presence of various important phytochemicals was marked, interestingly *Pterocarpus santalinus* showed a clear presence of Cardiac Glycoside when dissolved in methanol solvent, it was also observed that these plant samples have an immense capability to inhibit the microbial contamination and growth, especially when dipped and kept in an acidified water solvent. This is the first research that investigates the phytochemical properties and antibacterial activity of *Clitoria ternatea* and *Pterocarpus santalinus*. For performing the antibacterial property against *E. coli*, the agar well diffusion method was employed. When done against *E. coli*, methanol extract of Red Sandalwood produced the zone of inhibition with the largest diameter i.e., 25.5 mm, followed by acidified water extract of Aparajita of 23.0 mm and hydro alcohol extract of Aparajita about 11.5 mm. There is no zone

of inhibition for methanol extract of Aparajita and hydro alcohol, acidified water extract of Red Sandalwood.

Keywords: *Clitoria ternatea*, *Pterocarpus santalinus*, phytochemical screening, antimicrobial activity, Thin Layer Chromatography (TLC), *Rf* value.

Introduction:

Plants depend on inherent immunity to sense and adapt against possible pathogens because they lack a system of adaptive immunity like those observed in animal systems. To stop or reduce pathogen growth, innate immunity typically consists of two parts that activate cellular defence response pathways. Out of the two, this paper emphasizes the plant's innate immune system's initial line of defence. Microbe-associated molecular patterns (MAMPs, also known as pathogen-associated molecular patterns), wherein the molecular structures specific to microorganisms keep progressively changing and play a crucial role in microbial lifestyle, cause this reaction to be initiated. Detection of MAMPs like fungal chitin or bacterial flagellin can set off protective responses that promote plant immune response [1]. Since the beginning as we have known and noticed, harmful and disease-causing pathogens of any kind have developed diverse tactics to conquer any other organism, including plants and animals, most of these pathogens use the organism as a host to feed on and reproduce, this especially occurs in plants [2].

There are precursors of plants that are unadorned, and inorganic, that plants can use to generate a hefty multiplicity of organic compounds that has low molecular weight. This capacity of plants to generate organic compounds synthetically as per the need and situation helps them to lay claim to miscellaneous and challenging environments [3]. The vast majority of primary metabolite transporters exhibit a common feature of being substrate-specific facilitators, which allows them to speed up the diffusion of primary metabolites along a concentration gradient, or from high intracellular to low extracellular concentrations, across the plasma membrane via transmembrane carriers [4]. These substances are referred to as "secondary metabolites" because they are frequently low molecular weight, appear to have no direct impact on the plant's essential functions, and are typically low in molecular weight [5].

Kossel, who coined the term secondary metabolite more than a century ago, claimed that, in contrast to main metabolites, which are found in every cell of a plant that is able to divide, secondary metabolites are merely "accidentally" present and are not necessary for plant existence [6]. Other names for secondary metabolites are specialized metabolites and natural products. Secondary metabolites, many of which are lineage-specific and help plants interact with their biotic and abiotic environments, include important groupings such as phenolics, terpenes, and nitrogen-containing chemicals [7]. These metabolites have a long history of use as medications. The following categories of plant secondary metabolites are the most prevalent: terpenoids (cardenolides, diterpenoids, iridoids, monoterpenoids, sesquiterpenoids [including sesquiterpene lactones], and triterpenoids), coumarins, essential oils (lower terpenoids and phenylpropanoids), flavonoids, and steroids) [8].

There are four main categories of secondary metabolites: phenolic and polyphenolic compounds, around 8000 chemicals; sulfur-containing compounds and alkaloids, almost 12,000 compounds; and terpenoids, about 25,000 compounds. Additionally, these secondary metabolites primarily protect the body against sickness [9].

A well-known Ayurvedic remedy for a variety of diseases, *Clitoria ternatea L*. (CT), of the Fabaceae family, has undergone extensive scientific study. CT is also known as 'Bluebell Vine', 'Conch Flower', 'Shankapushpi', 'Butterfly Pea', 'Aparajita'. The Greek words kentron, which translates as a spur, prickle, sharp point, or the centre, and sema, which means a signal, are used to describe the spurred standard petal in the genus' scientific name. It is a beautiful perennial climber that may reach heights of two to three meters and grows both in the wild and in gardens. It has striking blue or white flowers that resemble conch shells **[10]**.

The plant's roots are made up of a somewhat thick taproot, a sparse branching structure, and numerous thin lateral roots. One to numerous purples, glaucous, wire stems are produced from the strong longitudinal root, which may attain a length of more than two meters **[11]**. Imparipinnate, five to seven leaflets, and 6 to 13 cm long leaves are an element of the plant. The leaflets are sub-coriaceous, ovate or oblong, and 2–5 cm long **[12]**. The leaflets' upper and lower epidermis have rubiaceous stomata with wavy cell walls. The subglobose or oval-shaped seeds have a yellowish-brown or black colour. In "Medhya Rasayana," a revitalizing concoction used to cure neurological diseases and believed to boost one's intelligence, CT has been used as an ingredient. Different therapeutic properties have been attributed to the roots, leaves, and seeds of CT in ancient medical systems that have been passed down orally or in writing (especially Ayurveda). The plant has been linked to numerous bioactive secondary metabolites and pharmacological effects **[13]**.

[Fig.1: Aparajita flower (*Clitoria ternatea*) and red sandalwood powder (*Pterocarpus santalinus*)]

Pterocarpus santalinus, frequently referred to as "Red Sanders" or "Red Sandalwood," is a prized forestry legume tree that is capable of reaching heights of as much as 8 meters and has a trunk that is 50 to 150 centimeters in girth. Red sandalwood trees begin to grow early in the growing season and can reach heights of up to 5 meters in just three years despite soils that have been depleted **[14]**. Red Sanders leaf and stem bark extracts show an extensive spectrum of activity over widespread human pathogenic bacteria, fungi, and protozoa. Red sander is known for its anti-inflammatory, analgesic, and anti-oxidant properties. Tannins, Flavonoids, Essential oils, Polyphenolic chemicals, and Phenolic acids all of which have a variety of biological functions, are present in the methanolic wood extract. When the livers of male CCl4-treated rats underwent research, the necrotized liver had higher levels of aspartate transaminase, alanine transaminase, serum bilirubin, and other enzymes, while having lower levels of total protein. Rats given Stem bark extract recovered significantly more than rats given no treatment **[15]**.

Pterocarpus, which refers to the winged pod, is derived from the Greek terms '*Pteron*' (wing) and '*Karpos*' (fruit), while *santalinus*, which refers to a plant with qualities resembling those of Indian sandalwood, Santalum album, is derived from the Latin 'Sandal' and '*Inus*' (meaning similar to). The

hard, dark-purple, bitter heartwood of *L. P. santalinus* is likewise highly valued. The pleasing aroma is brought on by the presence of 'Terpenoids', while, the colour and fragrance of *P. santalinus* heartwood are obtained from 'Santalins'. Red sandal heartwood gives out compounds termed as 'Benzofurans' demonstrated fatal dose-dependent cytotoxicity against Ca9-22 cancer cells [16]. It has recently been shown that these plants offer a variety of pharmacological activities, including anticancer, hepatoprotective, antibacterial, wound healing, and antidiabetic activities [17].

Materials and Methods:

SAMPLE COLLECTION:

Good and healthy samples of *Clitoria ternatea* and *Pterocarpus santalinus* were purchased and collected from the local market of Bhubaneswar, Odisha.

PREPARATION OF EXTRACT:

The fresh flower petals of *Clitoria ternatea* and dry bark powder of *Pterocarpus santalinus* and pulverizing was done. Separate small beakers were taken for each sample and 30 ml of distilled water, methanol, acidified water, and hydro alcohol were taken in these separate containers. Samples were immersed in these various solvents in small beakers that can be covered. The samples were incubated at normal room temperature for 24 hours [18]. The samples were filtered into separate beakers/tube bottles with the help of filter paper.

PHYTOCHEMICAL SCREENING:

Phytochemicals are extracted by different methodologies and are analyzed for their presence and absence by using different techniques and procedures. The presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins, tannins, and terpenoids can be tested qualitatively using the standard procedures to identify the constituents [19].

A. Test for Alkaloids (Wagner's Test):

Taken 1ml of plant extract and added 3-5 drops of Wagner's reagent and observed for the formation of reddish-brown precipitate or colouration, if positive.

B. Test for Carbohydrates (Molisch's Test):

Taking 1ml of plant extract and adding 3-5 drops of Molisch's reagent, along with this 1ml of concentrated sulphuric acid (H_2SO_4) was added to the side of the test tube. Then allowed the mixture to stand for 2-3 mins. Then observed the formation of red or dull violet colour at the interface of the two layers is a positive result.

C. Test for Cardiac Glycosides (Keller Kelliani's Test):

Taken 1ml of extract and treated with 1ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution. To that mixture added 0.5 ml of conc. H_2SO_4. Observed for a brown ring or violet ring at the interface which shows the presence of glycosides [20].

D. Test for Flavonoids (Alkaline Reagent Test):

1ml extract was treated with 3-5 drops of a 20% NaOH solution. The presence of flavonoids is indicated by the production of a bright yellow hue that becomes colourless after the addition of 0.5 ml dilute HCl.

E. Test for Phenols (Ferric Chloride Test):

Taking 1ml of extract and adding 5-6 drops of 5% aqueous ferric chloride solution and observed for the formation of deep blue or black colour.

F. Test for Amino acid and Proteins (Ninhydrin Test):

Taken 1 ml of extract and added 2-5 drops of Ninhydrin solution and kept it in a boiling water bath for 1-2 min and observed for the formation of purple colour.

G. Test for Saponins (Foam Test):

Take 1 ml of extract and added 5 ml of Distilled water and shake vigorously. Observed for the formation of persistent foam for 10-15 min that confirms the presence of saponins.

H. Test for Tannins (Braymer's Test):

1 ml of the extract was treated with 1 ml of 10% alcoholic ferric chloride solution and the production of blue or greenish hue was noticed.

I. Test for Terpenoids (Salkowski's Test):

Take 1 ml of extract and added 0.5 ml of chloroform along with 3-5 drops of conc. H_2SO_4. Observed for the reddish-brown precipitate produced immediately.

J. Test for Resins (Foam Test):

Take 1 ml of extract and added 5 ml of distilled water and observed for turbidity.

K. Test for Coumarins (Alkaline Test):

Taking 1 ml of extract and adding 1.5 ml of 10% NaOH then observed the formation of yellow colour which indicates the presence of coumarins.

CHROMATOGRAPHY:

Each of the plant extracts were checked by Thin Layer Chromatography (TLC) on analytical plates over silical gel. For each extract, the solvent system Benzene: Chloroform: Acetone = 3:1:1 was used as developing systems. In each case, the spots were visualized by naked eye and UV transilluminator [21].

ANTIMICROBIAL ACTIVITY FOR CRUDE EXTRACT:

The antibacterial activity of the generated antibiotic must be verified, which may be done using a variety of techniques. The most used technique is agar diffusion. For MIC determination in a solid medium, the agar diffusion method is frequently employed. It entails applying antibiotic solutions at various doses to paper discs, cups, or wells that have been drilled into or put on top of agar plates containing the test bacterial strain. As a result of antibiotic diffusion from these sources into the agarose medium, bacterial growth in the area around the source is inhibited and clear zones' devoid of bacterial lawn is created. With an increase in antibiotic concentration, these zones' diameters grow. The media was poured on the autoclaved petri plates, allowed them to solidify and then spread *E. coli* microbial strain and punched the wells followed by adding 0.1 ml of the crude extract to the wells, and incubated the plates for 24 hours at 37°C overnight in the incubator [22].

Results and Discussion:

PHYTOCHEMICAL SCREENING:

Two different plant samples viz., Aparajita, and Red Sandalwood were extracted by using four different solvents viz., Hydro-Alcohol (HA), Acidified Water (AW), Distilled Water (DW), and Methanol (M) by keeping them for up to 24 hours at room temperature.

Preliminary studies for the presence and absence of metabolites were carried out by different qualitative screening procedures which is shown in table.1 and table.2.

Phytochemicals	Plant Sample Extract			
	Aparajita (HA)	Aparajita (AW)	Aparajita (M)	Aparajita (DW)
Alkaloid	+ve	-ve	+ve	+ve
Carbohydrate	-ve	+ve	+ve	+ve
Cardiac glycoside	+ve	-ve	-ve	-ve
Flavonoid	-ve	-ve	-ve	-ve
Phenols	-ve	-ve	-ve	-ve
Protein	+ve	+ve	+ve	+ve
Saponin	-ve	+ve	-ve	-ve
Tannin	-ve	-ve	-ve	-ve
Terpenoid	+ve	-ve	+ve	-ve
Resin	-ve	-ve	-ve	-ve
Coumarin	+ve	+ve	+ve	+ve

[Table.1: Qualitative Screening of metabolites of different extracts of *Clitoria ternatea*]

Phytochemicals	Plant Sample Extract			
	Red Sandalwood (HA)	Red Sandalwood (AW)	Red Sandalwood (M)	Red Sandalwood (DW)
Alkaloid	+ve	-ve	-ve	-ve
Carbohydrate	-ve	+ve	-ve	-ve
Cardiac Glycoside	-ve	-ve	+ve	-ve
Flavonoid	-ve	-ve	-ve	-ve
Phenol	-ve	-ve	-ve	-ve
Protein	-ve	-ve	+ve	-ve
Saponin	+ve	+ve	-ve	-ve
Tannin	-ve	-ve	-ve	-ve
Terpenoid	-ve	-ve	-ve	-ve
Resin	-ve	-ve	-ve	-ve
Coumarin	-ve	-ve	-ve	-ve

[Table.2: Qualitative Screening of metabolites of different extracts of *Pterocarpus santalinus*]

THIN LAYER CHROMATOGRAPHY:

The TLC was carried out to observe separated molecules. Mobile phases were prepared with Benzene: Chloroform: Acetone of the ratio (3: 1: 1) respectively of the components. The Rf values are then measured and presented in the table.3 and table.4.

Extract ***(Clitoria ternatea)***	**Mobile Phase**	**Colour Observed**	***Rf***
Hydro Alcohol (AW)	Hydro Alcohol	Blue	0.89
		Yellow	0.60
	Acidified Water	Blue	0.50
		Light Green	0.78
	Distilled Water	Blue	0.69
Acidified Water (AW)	Hydro Alcohol	Blue	0.70
		Yellow	0.81
	Acidified Water	Blue	0.75
		Yellow	0.86
		Light brown	0.50
		Light pink	0.44
	Distilled Water	Blue	0.78
Methanol (M)	Hydro Alcohol	Blue	0.71
	Acidified Water	Yellow	0.83
	Distilled Water	Light green	0.71
Distilled Water (DW)	Hydro Alcohol	Dark blue	0.67
	Acidified Water	Light Purple	0.55
	Distilled Water	Light brown	0.85

[Table.3: Retardation factor for different extracts of *Clitoria ternatea*]

Extract ***(Pterocarpus santalinus)***	**Mobile Phase**	**Colour Observed**	***Rf***
Hydro Alcohol (HA)	Hydro Alcohol	Violet	0.59
		Orange	0.91
	Acidified water	Violet	0.27
		Yellow	0.92
	Distilled water	Orange	0.46
		Violet	0.69
		Yellow	0.92
Acidified Water (AW)	Hydro Alcohol	Violet	0.35
		Blue	0.81
	Acidified water	Violet	0.50
	Distilled water	Violet	0.76
Methanol (M)	Hydro Alcohol	1)Yellow	0.68
		2)Pink	0.34
	Acidified water	Yellow	0.92
		Violet	0.40
		Pale Blue	0.45
	Distilled water	Yellow	0.81

		Green	0.75
Distilled Water (DW)	Hydro Alcohol	Blue	0.34
	Acidified water	Violet	0.27
		Yellow	0.92
	Distilled water	Blue	0.69

[Table.4: Retardation factor for different extracts of *Pterocarpus santalinus*]

ANTIMICROBIAL ASSAY:

The crude extract was then subjected to antimicrobial screening. Both samples. i.e., Aparajita and Red Sandalwood showed a remarkable anti-microbial activity against *E. coli* when was soaked in Acidified Water.

Plant Sample	Zone of Inhibition (mm) of plant extracts by using different solvents against *E. coli*		
	Hydro Alcohol (HA)	Acidified Water (AW)	Methanol (M)
Aparajita	11.5	23.0	0
Red Sandalwood	0	0	25.5

[Table.5: Zone of inhibition (in mm) of *Clitoria ternatea* and *Pterocarpus santalinus* against *E. coli* bacteria]

The functional groups present in the methanolic extract of in vitro grown *C. ternatea* plants using AS and wild plants through FTIR analysis. In FTIR spectrum profile, increased peak value clearly evidenced that enhancement of functional groups such as alcohols, aldehydes, alkanes, amines, carboxylic acids, proteins, sulfonates, nitro compounds and halogenated compounds etc., when compared to the control plants. This is the first report in *C. ternatea* that the addition of adenine sulphate on regeneration medium enhances the phytocompounds (functional groups) quantity in in vitro propagated plants. From the statistical result, it was known that both Aparajita leaf extract and powder spray dried with maltodextrin as the carrier have the hypoglycemic effect [23].

An increase in HDL and reduction in LDL, TC has observed in combination of vitamin E and *P. santalinus*, when administered separately to each group of subjects. Body, muscle, heart, kidney and liver weight were all seen to benefit from the combinational approach. Weight loss due to excessive breakdown of tissue proteins is also a complication of Diabetes Mellitus. The combined treatment with *P. santalinus* and vitamin E showed promising results [24].

Conclusion:

Bioactive components contained in condiments are thought to be the best source for improving health prospects. These compounds can also be used to create prospective pharmaceutical molecules that could be developed into treatments for major diseases like cancer. The outcome of this investigation will reveal several molecules that are highly helpful for the production of new medications, most of which

will be organic and entirely plant-based. Due to the existence of phytochemical elements, the outcomes of the current investigations and prior phytochemical analyses are quite similar. 25 distinct plant species from the leguminosea family in total. Plant extracts from the Fabaceae family have been highlighted as potentially effective antibacterial agents.

While the chemical components of Aparajita's flower extract and Red Sandalwood's plant extract vary. In this study both were examined for possible antibacterial agents. The antimicrobial activity against *E. coli* showed the methanol extract of Red Sandalwood produced the zone of inhibition with the largest diameter i.e., 25.5 mm, followed by acidified water extract of Aparajita of 23.0 mm and hydro alcohol extract of Aparajita about 11.5 mm. There is no zone of inhibition for methanol extract of Aparajita and hydro alcohol, acidified water extract of Red Sandalwood. Interestingly characterization and separation of the chemically active elements present in these traditional plants also uncovered that *Pterocarpus santalinus* showed a clear presence of Cardiac Glycoside when dissolved in methanol solvent, the exact organic compound that plays a role in medicines for treating heart failure to measurable extents. In fact, both the plants showed the potential presence of various phytochemicals when immersed in the different types of solvents used as mentioned above. The extensive study for future investigation may result in the creation of potential medicines that may be used to treat a variety of illnesses and be fully embraced by the local population. The findings of this study might also be useful commercially to pharmaceutical companies and research organizations developing new medications.

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Conflict of Interest:
Nil

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