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PHYTOCHEMICAL AND ANTIMICROBIAL SCREENING OF INDIGOFERA TIRUNELVELICA SANJAPPA: AN IMPORTANT HERB

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ABSTRACT

The therapeutic value of *Indigofera tirunelvelica Sanjappa* (Fabaceae) has been recognized as a component of traditional medication for the treatment of various human ailments. The plant is being highly explored, still lacks sufficient evidences for the best variety possesses the highest degree of medicinal values. The present study is focused on phytochemical screening of aqueous and ethanolic leaf extracts of *I. tirunelvelica*. The crude extracts of *I. tirunelvelica* revealed the presence of several biologically active phytochemicals with the high degree of alkaloids, flavonoids and phenols. The antibacterial efficacy was investigated against pathogenic bacterial strains and the highest inhibitory activity of aqueous extract was obtained against *Staphylococcus aureus*, *Salmonella typhi* and *Actinomyces* whereas ethanolic extract was found to be most potent against *Klebsiella*, *Pseudomonas auroginsoa*, *Staphylococcus aureus* and *E.coli* at 50 µg/ml concentration. The preliminary phytochemical screening revealed the presence of many bioactive metabolites such as flavonoids, terpenoids, phenolics, and steroids that can be postulated for antibacterial activity.

KEYWORDS: *I. tirunelvelica*; Ethanol extract, medicinal plant, and antimicrobial activity.

INTRODUCTION

Novel drug discoveries have budged attention from synthetic models and compounds to natural derivative of medicinal plants because it is understood that drugs from medicinal plants are safe, free from side effects. WHO (2002) proclaimed that the 80% of world people

is still reliant on herbal medicines to treat their diseases.[1] In the recent situation, the demand for herbal products is growing exponentially throughout the world, and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal value. This is due to believing of scientists that ligand/leads discovery would be more possible in medicinal plants and which are yet to be fully investigated.[2] Therefore, quality control for the efficacy and safety of herbal products is essential. In India, the primary commitment of department of AYUSH (Ayurvedic, Unani, Siddha, Homeopathy, under ministry of health and welfare, Government of India) is to deal with the rules and regulations for the herbals along with the Drugs and Cosmetic Act and Rules for the implementation of Good manufacturing practices (GMP) in herbals. The steps are taken by this department not only help to make the quality of herbal products but also to safeguard the adverse effects of the herbals too.[3] *Indigofera tirunelvelica* Sanjappa (Fabaceae) is an annual erect herbs, about 60 cm high, branches woody, angular, light brown pubescent when young terete, striate and glabrous at maturity. Leaves 3,5.4 cm long, pinnately trifoliolate, alternate; petioles 1-.3 cm long, slender, canaliculated above., Flowers pink, 5mm long; pedicels short, pubescent, glandular; bracts 1-1.5 mm long, lanceolate, acute, pubescent without, caducousl cayx 2mm long, 5 – lobes, lobes, 1-1.5 mm long. Flowering during November to December months. And fruiting is during December to march. *Indigofera tirunelvelica* distributed in and around Tirunelvel Hills, Tamil Nadu.[4] There are no reports about its chemical constituents and pharmacology, safety and uses until now. At first time, we present study report that includes organoleptic characteristics, Fluorescence analysis, physicochemical analysis, preliminary phytochemical screening, quantitative phytochemical analysis and antimicrobial activity of *I. tirunelvelica*.

MATERIALS AND METHODS

The fresh leaves of *Indigofera tirunelvelica* Sanjappa were collected from Thirunelveli District, Tamil Nadu, India.

Determination of foreign matter

Accurately weighed 250 g of the leaf material was spread in to thin layer and the foreign matter was sorted into groups by visual inspection, using a magnifying lens. The remaining sample was shifted through a number 250 sieve; dust was regarded as mineral admixture. The content of each group was calculated in grams per 100 g of air-dried sample.[5]

Organoleptic Evaluation

The organoleptic characters of the samples were evaluated based on the method described by Siddiqui *et al*. Organoleptic evaluation refers to evaluation of the formulation by color, odor, taste and texture etc.[6]

Fluorescence Evaluation

Fluorescence of the powder was observed under day and UV light (254nm) treating with acids and alkaline solutions of the drug.[7]

Physiochemical Evaluation

Physiochemical studies are evaluated to determine the quality and purity of the leaf powder of *I. tirunelvelica*. The parameter was done to evaluate the percentage of moisture content (Loss of dry), total ash, water soluble and acid insoluble ash were calculated as per Indian Pharmacopoeia.[8,9] The extract of the powdered leaves were prepared with the different solvents for the study of extractive value. Fluorescence analysis was also carried out for the powder and also with extracts with different solvents (Hexane, Chloroform, Ethyl acetate, Ethanol and Water).

Preliminary Phytochemical screening

The leaf powder of *I. tirunelvelica* and extraction from different solvents (Hexane, Chloroform, Ethyl acetate, Ethanol and Water) were studied for the presence and absence of secondary metabolites like, alkaloids, glycosides, saponins, phytosterols, phenolics, terpenoids, flavonoids, coumarins, steroids, sugars, quinines. lignin, starch, protein and tannins by qualitative chemical tests.[10,11]

Antimicrobial study

The antimicrobial activities of *I. tirunelvelica* were carried out by disc diffusion method.[12] The bacterial organisms like *Actinobacteria*, *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mitis*, *Streptococcus mutans*, *Enterobacter aerogenes*, *Klebsiella pneumonia*, *Pseudomonas aeroginosa*, *Salmonella typhi* and *Escherichia coli* were swabbed over the prepared nutrient agar plates by using separate sterile cotton buds. Then the fungal test organism like *Aspergillus candidtis*, *Nieospora oryzae*, *Aspergillus niger*, *Alternaria brassicae* and *Helminthosporium solani* were spread over the potato dextrose agar plates. After the microbial lawn preparation, the sterilized disc was dipped in 50 µg/ml concentrations of each extracts of *I. tirunelvelica* placed on the organism inoculated plates

with equal distance. Streptomycin (10 mcg) antibacterial disc and Amphotericin B (20 mcg) antifungal discs were acted as positive control for bacteria and fungus respectively and different solvents were acted itself as a negative control. All bacterial plates were incubated at 27°C for 24hrs and fungal plates at 24°C for 72hrs. The diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed.

Statistical Analysis

All experiments were repeated at least three times. Results are reported as Mean ± Standard deviation.

RESULTS AND DISCUSSION

As a part of standardization procedure, the leaf sample of *I. tirunelvelica* was tested for relevant physiochemical, phytochemical parameters and also subjected to antimicrobial activity, cytotoxicity screening through quality control measures.

Determination of foreign matter

The foreign matters which are other than our study plant material such as any organism or product of an organism and mineral admixtures not adhering to the medicinal plant materials for example soil, stones sand and dust. This foreign matters present in medicinal plant materials may interfere throughout the study, it is difficult to examine them. However, microscopy is indispensable for powdered materials. Table 1 showed the 2.21±0.67% w/w of total foreign matter which showed the possibility of entirely free from some form of foreign matter.

Organoleptic Evaluation

The Organoleptic studies indicated that important characteristic such as typical tongue sensitizing chemical taste, and different characteristic odour which are useful diagnostic characters. Organoleptic parameters of *I. tirunelvelica* revealed that the powder of leaves were green in color, with an aromatic odor and bitter in taste (Table 2).

Fluorescence Evaluation

The fluorescence characters of powdered drug and extracts of different solvents play a vital role in the determination of quality and purity of the drug materials. Fluorescence properties of powdered leaves (Table 3) and various extracts (Table 4) were studied. The powder showed fluorescence with various chemical reagents and extracts of different solvents also

exhibited fluorescence properties. Some constituents show fluorescence in the visible range in many natural products which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.

Physiochemical Evaluation

The determination of physiochemical parameter is important to determination of adulterants and improper handling of drugs. Table 4 showed total ash content was 5.66±0.24% (w/w). The amount of acid insoluble ash and water soluble ash were found to be 0.75±0.07 and 5.10±0.65% (w/w) respectively. Ash values are useful in determining the quality and purity of crude drugs in powdered form. The total ash usually consists of inorganic radicals like carbonates, phosphates, silicates and silica of sodium, potassium, magnesium and calcium. Sometimes, inorganic variables like calcium oxalate, silica, carbonate content of crude drug affects "total ash" values, such variables are then removed by treating with acid (as they are soluble in HCl) and then acid insoluble ash value is determined. The values vary within fairly wide limits and are therefore an important parameter for the purpose of evaluation of crude drugs. Ash insoluble in HCl is the residue obtained after extracting the total ash with HCl. This acid insoluble ash value particularly indicates contamination with silicious materials like earth or sand. Here, Low acid insoluble ash (0.75±0.07) indicates less silicious materials like earth or sand. Water soluble ash is that part of the total ash content which is soluble in water. It is a good indicator of either extraction of water soluble salts in the drug or incorrect preparation. While determining the total ash, very high temperature (> 650°C) may result in the conversion of carbonates to oxides. Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug.[13] The moisture of content (Loss on drying) of *I. tirunelvelica* leaves is found to be 0.61±0.07% w/w (Table 4). Loss on drying determines both water and volatile matter. Excess of water in medicinal plant materials will encourage microbial growth and deterioration following hydrolysis. This is especially important for materials that absorb moisture easily or deteriorate quickly in the presence of water. The less value of moisture content could prevent bacterial, fungal or yeast growth.

The extractive value of the crude drug determines the quality as well as purity of the drug.[14] In present study, Hexane, Chloroform, Ethyl acetate, Ethanol and Water of leaves of *I.*

tirunelvelica extractive values were determined. Extractive values of *I. tirunelvelica* leaves showed very high quantity of polar constituents than non-polar constituents (Table 5). Extractive values determine the amount of active constituents extracted with solvents from a given amount of medicinal plant material and are useful for evaluation of crude drugs and give an idea about the nature of chemical constituents present in them. The amount of extractive drug yield to a given solvent is often an approximate measure of a certain constituent or group of related constituents the drug contains. In some cases the amount of drug soluble in a given solvent is an index of its purity. The solvent used for extraction should be in a position to dissolve appreciable quantities of substances desired. Taking into consideration the diversity in chemical nature and properties of contents of drugs, various solvents are used for determination of extractive values. These experiments were repeated thrice in order to arrive at standard values. The results showed greater extractive values in water, ethanol, ethyl acetate, chloroform and hexane extractions indicating the effect of chemical compounds present in the plant.

Qualitative Phytochemical screening

Preliminary Phytochemical results showed in Table 7 indicated the presence or absence of certain phytochemical in the drug and also needed to standardize the crude drugs and it's become very important for identification and authentication of drug. The leaves powder of *I. tirunelvelica* showed that the presence of Alkaloids, Phenol, Glycosides, Flavones, Steroids, Starch, Terpenoids and Protein in it. With extracts, Phenol, alkaloids and Glycosides were detected in all leaf extracts of *I. tirunelvelica*. Flavones present in all extracts except hexane extracts, likewise steroids present in all the extracts but ethanol extracts. Starch was found in ethylacetate, ethanol and water extracts. Aqueous and ethanolic extracts have terpenoids in it. Comarin was found in hexane, ethyl acetate and ethanol extracts. None of the extracts has tannin, saponin, lignin, protein and quinone in their extracts. Results obtained from phytochemical analysis could make the plant useful for treating different ailments and having a potential of providing useful drugs for human use and further work is required to investigate the leaf extracts of *I. tirunelvelica* for various pharmacological activities.

Antimicrobial Evaluation: Antibacterial and Antifungal study

In the present investigation, the inhibitory effect of different extracts (Water, Ethanol, Chloroform, Ethyl acetate and Hexane) of *I. tirunelvelica* were evaluated against in both

bacterial and fungicidal strains. The growth inhibitory effects of tested pathogens were determined using the disc diffusion method and the results showed in Table 8.

All the tested pathogens are highly susceptible to the crude extracts. However, our study revealed a remarkable antibacterial activity against gram-negative bacterial strains than gram-positive at 100 µg concentrations. The ethanol, water, chloroform have showed maximum zone of inhibition but Ethyl acetate and hexane extracts revealed moderate inhibitory activity against all tested bacteria as showed in Table 8. Similar with the bacterial result, the activity against fungal organisms showed maximum zone of inhibition by ethanol, water, chloroform extracts at 100 µg concentrations. However, Ethyl acetate and hexane extracts showed mild inhibitory activity against all tested organisms as showed in Table 8.

Table 1: Determination of foreign matter of Leaves of *Indigofera tirunelvelica.*

S.No.	Characteristics	% w/w
1.	Foreign matter	2.21±0.67

Table 2: Organoleptic characteristics of leaves powder of *Indigofera tirunelvelica.*

S.No.	Chararcteristics	Observation
1.	Appearance	Powder
2.	Colour	Green
3.	Odour	Aromatic
4.	Taste	Bitter

Table 3: Fluorescence analysis of leaves powder of *Indigofera tirunelvelica.*

Powder + Chemicals	After 24h		After 48h	
	Normal light	UV light	Normal light	UV light
Powder	Bluish green	Green	Bluish green	Green
Powder + 50% H_2SO_4	Black	Brown	Black	Dark Brown
Powder + Aq.1N NaOH	Light Green	Yellow	Dark green	Green
Powder + $CHCl_3$	Light Blue	Transparent	Blue	Blue
Powder + Ethylacetate	Brown	Green	Dark Brown	Dark green
Powder + Hexane	Green	Orange	Green	Orange
Powder + Acetone	Light Blue	Transparent	Blue	Blue
Powder +Benzene	Orange	Green	Red	Dark Green
Powder + 1N HCl	Green	Transparent	Dark Green	Light brown
Powder + Alc. 1N NaOH	Light Green	Pale yellow	Green	Yellow
Powder + Alcohol	Light Green	Yellowish Red	Green	Red
Powder + Water	Blue	Light green	Dark blue	Brownish green

Table 4:- Fluorescence Analysis of different leaves extracts of *Indigofera tirunelvelica.*

Extracts of Different solvents	After 24h		After 48h	
	Normal light	UV light	Normal light	UV light
Hexane	Dark green	Red	Dark green	Dark red
Chloroform	Light Blue	Dark Blue	Blue	Dark Blue
Ethyl acetate	Brown	Green	Dark Brown	Dark green
Ethanol	Green	Dark red	Green	Dark red
Water	Blue	Green	Blue	Dark green

Table 5: Determination of Ash Values of leaves powder of *Indigofera tirunelvelica.*

S.No	Parameters	Value % (W/W)
1.	Total Ash	5.66±0.24
2.	Acid Insoluble Ash	0.75±0.07
3.	Water Soluble Ash	5.10±0.65
4.	Moisture content	0.61±0.07

Table 6: Determination of Extractive Value of leaves powder of *Indigofera tirunelvelica.*

S. No.	Extracts of Different solvents	Value % (W/W)
1.	Hexane	0.84±0.19
2.	Chloroform	2.05±0.09
3.	Ethyl Acetate	4.37±0.29
4.	Ethanol	7.76±0.16
5.	Water	25.89±0.14

Table 7: Preliminary phytochemical investigation of different extracts of leaves powder of *Indigofera tirunelvelica.*

S. No.	Phytochemicals	Powder	Extraction of different solvents				
			Hexane	Chloroform	Ethyl acetate	Ethanol	Water
1	Terpenoids	+	-	-	-	+	+
2	Flavones	+	-	+	+	+	+
3	Steroids	+	+	+	+	-	+
4	Alkaloids	+	+	+	+	+	+
5	Phenol	+	+	+	+	+	+
6	Tannin	-	-	-	-	-	-
7	Saponin	-	-	-	-	-	-
8	Coumarin	-	+	-	+	+	-
9	Lignin	-	-	-	-	-	-
10	Protein	+	-	-	-	-	-
11	Glycosides	+	+	+	+	+	+
12	Quinone	-	-	-	-	-	-
13	Starch	+	-	-	+	+	+

+ ve indicates Presence.

- ve indicates Absence.

Table 8: Zone of inhibition by different extracts of *Indigofera tirunelvelica.*

S. No.	Bacteria	Water (mm)	Ethanol (mm)	Chloroform (mm)	Ethyl acetate (mm)	Hexane (mm)
1.	*Klebsiella*	11.23±0.25	13.00±0.87	11.50±0.50	6.72±0.30	10.77±0.40
2.	*Pseudomonas auroginsoa*	11.10±0.10	13.27±0.31	11.40±0.53	10.37±0.32	9.57±0.38
3.	*Staphylococcus aureus*	14.77±0.38	14.97±0.06	12.83±0.35	10.93±0.40	7.51±0.17
4.	*E.coli*	11.30±0.62	14.43±0.51	12.70±0.26	7.46±0.47	7.22±0.20
5.	*Enterococcus aerogenus*	10.93±0.31	11.80±0.44	11.63±0.15	10.63±0.25	6.33±0.21
6.	*Salmonella typhi*	12.83±0.49	9.73±0.31	10.80±0.20	11.43±0.12	7.44±0.48
7.	*Bacillus subtilis*	11.23±0.25	12.53±0.12	10.63±0.32	7.68±0.36	11.27±0.40
8.	*Streptococcus mutant*	11.53±0.31	12.77±0.25	9.23±0.21	7.80±0.36	7.52±0.33
9.	*Streptococcus mytise*	11.77±0.25	12.70±0.17	8.83±0.29	6.85±0.53	7.27±0.06
10.	*Actinomyces*	12.37±0.32	11.20±0.75	9.36±0.27	8.23±0.32	7.57±0.21
11.	*Aspergillus candidtis*	11.60±0.26	12.23±0.21	8.67±0.31	8.31±0.13	8.88±0.54
2.	*Nieospora oryzae*	10.43±0.51	11.38±0.19	10.23±0.38	7.50±0.10	6.77±0.39
13.	*Aspergillus niger*	11.40±0.72	11.90±0.36	11.20±0.52	7.27±0.26	7.46±0.34
14.	*Alternaria brassicae*	12.23±0.60	11.00±0.35	11.20±0.10	10.73±0.59	9.63±0.18
15.	*Helminthosporium solani*	11.67±0.42	12.40±0.10	11.67±0.40	9.26±0.72	9.90±0.40

CONCLUSION

In conclusion, the physicochemical and antimicrobial analysis of leaves of *Indigofera tirunelvelica* provides substantial information for the proper identification, authentification, and scientific evaluation of the drug. The present study results showed the phytochemical and its antimicrobial activity of ethanol and aqueous extracts of *I. tirunelvelica* which exhibited a significant antibacterial activity, suggesting the presence of either good antibacterial potency or the high concentration of the active principle in the extracts. This simply means the plant exhibit moderate antimicrobial activity. The observed tendency of the extract to inhibit Gram-negative bacteria more than the Gram-positive at low concentrations showed that *I. tirunelvelica* contain interesting bio-medicinal substances capable of attracting significant scientific attention. This study also showed that *I. tirunelvelica* exhibited phytochemical and antibacterial activities of natural origin which may offer promising antibacterial agents. Investigation will be carried on the *in vitro* antioxidant, pharmacologic activity of the plant and compounds responsible for these activities to be elucidated.

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