EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 8
Issue: 9
525-532
Year: 2021

PHYTOCHEMICAL ANALYSIS OF *TECAMA STANS* PRELIMINARY SCREENING OF IN-VITRO ANTIMICROBIAL ACTIVITY AND ANTIOXIDANT POTENTIAL

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Article Received on 14/07/2021 **Article Revised on 03/08/2021** **Article Accepted on 23/08/2021**

ABSTRACT
Fundamental screening of phytochemicals is a significant advance, in the recognition of the bioactive standards present in restorative plants and therefore, may prompt medication disclosure and improvement. In the current investigation, boss phytoconstituents of the *Tecama stans* restorative plant of Bignoniaceae family were recognized to relate their essence with bioactivities of the plants. These exploration discoveries feature that methanolic concentrates of *Tecama stans* had the most elevated number of phytochemicals contrasted with other dissolvable concentrates. Thus, ethanolic concentrates of *Tecama stans* holds the extraordinary potential to treat different human infections and has significant clinical pertinence.

KEYWORDS: *Tecama stans* Physiochemical; Phytochemical Screening Antimicrobial activity and Antioxidant potential UV, FTIR.

1. INTRODUCTION
Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population. Almost all the plants of *Tecoma stans* are of medicinal importance and used traditionally for the treatment of various ailments.[1] The plants are leaves, flowers, barks and roots have been used as chloretic activity, mild cardiotonic and muscle relaxant. Pharmacological uses *Tecoma stans* have been used in herbal medicine treatment for reducing blood glucose,[2] Plants have been used for the treatment of several diseases for so many years now before the introduction of orthodox drugs which have their bioactive constituents synthesized from plants with modifications. Antibacterial, antifungal, antimicrobial, and anticancer properties are all found as a result of the secondary metabolites of plants.[3,6]

Plant is in use through Mexico, India and Central America for diabetes, roots for diuretic and urinary disorder control. *Tecoma stans* was also investigated for antifungal effect in roots. Standardization of a plant is first requirement for its use in herbal medicines.[7] control of *Tecoma stans* have been found in treating diabetes and digestive problems.[10] The Literature survey reveals that the Tecoma yeast infections, as powerful diuretic activity, vermifuge and tonic.[8] Flower and leaves have some medicinal value for the treatment of variou cancer.[9] The primary applications of stans possesses various bioactive compounds such as flavonoids, alkaloids, steroids, saponins, tannins, phyto sterols, triterpenes, phenols and glycosides.[11,12]

2. MATERIALS AND METHODS
Collection and Identification of the Plant Material
The flower of *Tecama stans* were gathered from the Poondi, Thanjavur District, Tamil Nadu. The plant was related to help of nearby vegetation and verified in Botanical Survey of India, Southern Circle, Trichy, Tamil Nadu.

Preparation of *Tecama stans* Powder
Dry conditions are fundamental to forestall the development of antiques because of microbial maturation and resulting corruption of the plant metabolites. The plant materials are cut or cut into little pieces to work with homogenous drying and kept from direct daylight effect on limit unwanted substance responses of plant metabolites bringing about the arrangement of antiques. Henceforth in the current investigation, the leaves of *Tecama stans* were cut into little pieces and were dried in shade and afterward powder with a mechanical processor. The powder was going through sifter number 75 and put away in a marked hermetically sealed holder for additional examinations.

Physicochemical Studies
Physicochemical investigations remember misfortune for drying (dampness content), debris esteem and extractive worth to decide the quality and immaculateness of the powder of the flower of *Tecama stans*.

Moisture (Loss on Drying)
About 3g of the air-dried example was gauged (Wb), into a pre-dried and gauged (Wa) tarred porcelain cauldron. The example was dried in a stove at 100-105°C until two successive gauging's (Wc) don't contrast by more than 5mg. The dampness content of the example was determined concerning the rough air-dried medication.

Ash Values
Total Ash Value
A silica pot was warmed to redness for 10min and cooled in a desiccator and gauged (W1). Around 3 g of the ground air-dried example was moved to the pot and weighed alongside the substance precisely (W2). The example was lighted progressively in an electrical stifle heater, expanding the warmth to 500–600°C until it is white, demonstrating the shortfall of carbon. It was cooled in desiccators and rechecked (W3).

Acid-insoluble Ash(Silica & Sand content)
10 ml of 2 M HCl was added to the cauldron containing the complete debris, covered with a watch-glass and bubbled tenderly for 5 minutes. The watch-glass was washed with 5 ml of heated water and the washings were added to the cauldron. The insoluble matter was separated on an ashless channel paper and washed with heated water until the filtrate is unbiased. The channel paper containing the insoluble matter was moved to the first cauldron, dried on a hotplate and touched off to consistent weight (W4).

Water Soluble Ash
To the pot containing the absolute debris, 25 ml of water was added and bubbled for 5 minutes.[13] The insoluble matter was gathered on an ashless channel paper. The channel was washed with boiling water and afterward lighted in a pot for 15 minutes at a temperature not surpassing 450°C. The buildup was permitted to cool in desiccators for 30min, and afterward re-gauged (W5), estimations were finished by conditionsWeight of residue, W6 (g) = W5 – W1
Weight of ash W7 (g) = W3 – W1
Water-soluble ash (g) = W7 – W6

Sulphated Ash
A silica cauldron was warmed to redness for 10 minutes, permitted to cool in desiccators and gauged (Wa). 1g of substance was precisely gauged and moved to the cauldron and weighed alongside the substance precisely (Wb). It was touched off delicately at first until the substance was completely burned. Then, at that point the buildup was cooled and soaked with 1 ml concentrated sulfuric corrosive, warmed tenderly until white vapor are presently not developed and touched off at 800 ± 25°C until all dark particles have vanished. The start was led in a spot shielded from air flows.[14,18] The cauldron was permitted to cool, and a couple of drops of concentrated sulfuric corrosive were added and warmed. Touched off as in the past, permitted to cool, and gauged (Wc). The activity was rehashed until two progressive weighing doesn't vary by more than 0.5 mg.

Extractive Values
The extractive upsides of flower of *Tecama stans* in different solvents like oil ether, benzene, chloroform, ethyl acetic acid derivation, ethanol, methanol and water were controlled by utilizing the strategy for investigation depicted in Pharmacopeia of India.

Around 5 g of air-dried leaf powder was taken in a stoppered jar. 100 ml of the particular dissolvable was added, shaken well and permitted to represent 24 h with intermittent shaking. Then, at that point the substance was sifted. 50 ml of the filtrate were pipette out into a clean, recently gauged china dish and vanished on a water shower. At last, it was dried at 105ºC in a stove, cooled in a desiccator and weighed.[19] The level of dissolvable solvent extractive concerning the air-dried example was determined.

Preliminary Phytochemical Screening:
Preparation of Plant Material
The flower of the *Tecoma stans (Family: Bignoniaceae*) were collected, washed, shade dried and powdered. The powder was preserved in air sealed polythene cover for further evaluation.

Preparation of Plant Extract
The dried powdered tubers were defatted with ethanol (30 to 40 ºC) by hot extraction method in a soxhlet apparatus. The defatted powder materials were further extracted with ethanol and concentrated extracts were used for the analysis.

Phytochemical Screening
The phytochemical screening gives an overall thought in regards to the presence of various mixtures having remedial qualities. The diverse dissolvable concentrates of *Tecama stans* leaf were utilized for screening the presence of alkaloids, steroids, coumarin, tannins, saponins, flavonoids, quinone, anthraquinone, phenol, protein, xanthoprotein, starch, glycosides, catechin, sugar and terpenoids as per standard methodology.

Evaluating for Alkaloids (Dragendroff's test)
2 ml of the concentrate was blended in with 8 ml of 1% HCl, warmed and sifted. Then, at that point the filtrates were treated with Dragendroff's reagent (arrangement of Potassium Bismuth Iodide). Arrangement of a red accelerate demonstrates the presence of alkaloids.

Evaluating for Steroids (Liebermann Burchard test)
Concentrates were treated with chloroform and separated. The filtrates were treated with not many drops

of acidic anhydride, bubbled and cooled. Concentrated sulphuric corrosive was added. Development of the earthy colored ring at the intersection demonstrates the presence of phytosterols.

Evaluating for Coumarin
2 ml of the concentrates was taken in test tubes. The mouth of the cylinder was covered with channel paper treated with 3 ml of 1 N NaOH arrangement. The test tube was set for a couple of moments in bubbling water and afterward the channel paper was eliminated and inspected under the UV light for yellow fluorescence showed the presence of coumarins.

Evaluating for Tannins
50 mg of different dissolvable concentrate powder was broken up in 10 ml refined water and separated. 1% watery iron chloride (FeCl3) arrangement was added to the filtrate. The presence of exceptional green, purple, blue or dark shading demonstrated the presence of tannins in the test tests.

Evaluating for Saponin
50 mg of the different dissolvable concentrate powder was bubbled in refined water in a test tube in bubbling water shower and sifted. 10 ml of the filtrate was blended in with 5 ml of refined water and was shaken vivaciously to the arrangement of stable constant foam. The foaming was blended in with 3 drops of olive oil and shaken vivaciously for the arrangement of emulsion consequently a trait of saponins.

Evaluating for Flavonoids (Shinoda Test)
To the concentrate arrangement (5 ml), added not many parts of magnesium lace and thought HCl dropwise. The presence of red or orange-red tone demonstrates the presence of flavonoids.

Evaluating for Quinone
1 ml of the concentrate was blended in with 1 ml of concentrated H2SO4. The presence of red shading shows the presence of Quinone.

Evaluating for Anthroquinone (Borntrager's test)
50 mg of concentrate powder was taken into a dry test cylinder and 5 ml of chloroform was added and shaken for 5 minutes. The concentrate was sifted through Whatman No 1 channel paper and the filtrate was shaken with an equivalent volume of 10% smelling salts arrangement. A pink violet or red tone in the ammoniacal layer (lower layer) shows the presence of anthraquinone.

Evaluating for Phenols
The concentrate powder (50 mg) was broken up in 5 ml of refined water. To this couple of drops of 10% ferric chloride arrangement was added. The presence of a blue or green tone demonstrates the presence of phenol compounds.

Evaluating for Protein
The concentrate powder (50 mg) was broken up in 10 ml of refined water and sifted through Whatman No. 1 channel paper. To the filtrate, 1 ml of 40% NaOH was added. Then, at that point, 1 or 2 drops of 2% copper sulfate arrangement were added. The presence of violet tone demonstrates the presence of proteins.

Evaluating for Carbohydrates (Molisch Test)
To 2 ml of concentrates, 3 drops of α-naphthol (20% in ethanol) was added. Then, at that point 1 ml of concentrated sulphuric corrosive was added at the edge of the test tube. Ruddy violet ring at the intersection of the two layers showed the presence of sugars.

Screening for Glycosides (Borntrager's test)
Extract powder (50 mg) was mixed with concentrated H2SO4 (5 ml.), then it was heated for 3 minutes, thereafter it was filtered after that filtrate was mixed with 0.5 ml of 10% NaOH and allowed to stand for 3 minutes. The appearance of a reddish-brown precipitate indicates the presence of glycosides.

Screening for Reducing Sugar
For the presence of reducing sugars in the extract, Fehling test was performed. An amount of 50mg of the extract powder was taken and added it to the equal volume of boiling Fehling solutions (A and B) in a test tube. A brick- red precipitates indicate the presence of reducing sugar.

Screening for Terpenoids (Salkowski test)
5 ml of the various solvent extract was mixed in 2 ml of chloroform followed by the careful addition of 3 ml concentrated sulfuric acid (H2SO4). A layer of the reddish-brown colouration was formed at the interface thus indicating a positive result for the presence of terpenoids.

UV Fluorescence analysis
Take about 0.5 g of flower powder into clean and dried test tubes. To each tube 5 ml of different organic solvents like distilled water, 1N HCl, glacial acetic acid, 1N HNO3, liquid ammonia, 5% Ferric chloride, 5% iodine, methanol, petroleum ether, chloroform, 50% sulphuric acid, 40% NaOH and lead acetate were added separately. Then, all the tubes were shaken and they were allowed to stand for about 20-25 min. The solutions obtained were observed under the visible daylight and UV light of short wavelength (254 nm) and UV light of long-wavelength (365 nm) for their characteristic colour.

Determination of Antioxidant Activity
DPPH Assay
Free radical scavenging activity of different extracts is tested against a methanolic solution of 1-1- diphenyl-2picryl hydrazyl (DPPH). Antioxidants react with DPPH and convert it to 1-1- diphenyl-2picryl hydrazine. The degree of discoloration indicates the scavenging potential of the antioxidant extract. The change in the

absorbance produced at 517 nm has been used as a measure of antioxidant activity.

The sample of different extract is prepared in various concertration viz. 25, 50, 75, 100, 125 µg/ml in AR grade Methanol. 1 ml samples of above concertrations are mixed with equal volume of 0.1 mm Methanolic solution of DPPH (0.39 mg in 10 ml methanol). An equal amount of methanol and DPPH is added and used as a control Ascorbic acid solution of various concertration viz. 25, 50, 75, 100, 125 µg/ml in distilled water are used as standard. After incubation for 30 minutes in dark absorbance is recorded at 517 nm. Experiment is performed in triplicates. Percentage scavenging is calculated by using the following formula11.

Calculation

Percentage of anti-radicals activity = (A-B/A) x 100
Where A= Absorbance of control.
B= Absorbance of sample.

UV and FT-IR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FT-IR spectrophotometer analysis, the extracts were centrifuged at 3000 xg for 10 minutes and filtered through Whatmann No. 1 filter paper. The sample was diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks in a range between 400-4000 cm-1 and their functional groups. The peak values of the UV and FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

3. RESULTS AND DISCUSSION

Plants synthesize a wide array of compounds that play key roles in protecting plants against herbivores and as attractants for pollinators and seed-dispersing animals, allelopathic agents, UV protectants, and signal molecules in the formation of nitrogen-fixing root nodules in legumes. Although they have long been ignored from a nutritional perspective, the function of these compounds and their relative importance to human health are gaining significant interest. Therefore, the present study was to investigate the phytochemicals and antioxidant activities of *Tecoma stans* flower.

Table 1: Data on organoleptic characters of *Tecoma stans* Flower powder.

Character	*Tecoma stans* flower powder
Colour	Yellow
Taste	Tastless
Odour	Aromatic
Texture	Soft

The Various organoleptic characteristics of the plant *Tecoma stans* flower powder and the details are shown in Table 1. The preliminary observation on the organoleptic characteristics of the study plant form the base line investigations into any herbal drug study and the details shown here are self-explanatory. These parameters will be useful for standardization purpose of plant drug.

Table 2: Data on Fluorescence studies of *Tecoma stans* Flower powder.

Characters	*Tecoma stans* flower powder	
	Day light	U V light at 365 nm
Powder as such	Brown	Green
Powder + 1N sodium hydroxide	Light Brown	Dark Green
Powder + 1N hydrochloric acid	Yellow	Green
Powder + 50% sulphuric acid	Brown	Green
Powder + conc. nitric acid	Yellow	Light Green
Powder + ferric chloride	Green	Green
Powder + ammonia	Yellow	Green
Powder + sodium nitro preside	Yellow	Light Violet
Powder + 5% potassium hydroxide	Brown	Green
Powder + picric acid	Yellow	Dark Green
Powder + acetic acid	Dark Yellow	Dark Green

The fluorescence characteristics of *Tecoma stans* flower powder which is self-explanatory. It is to be noted that flower powder when treated with various analytical reagents yielded different colours. The colours are observed in day light and UV light at 365 nm.For example in sodium nitropruside it gives yellow color in daylight and light violet in UV at 365 nm.This range of wave length has been fixed by earlier workers for fluorescence studies, possible conventional way. Due totheir display of fluorescent characteristics of plant drug at this wavelenth are used for assessing the purity of raw drug samples.

Table 3: Physicochemical characteristics of *Tecoma stans* Flower powder.

Characters	*Tecoma stans* Flower(g)
Total ash	0.204
Water soluble ash	0.054
Acid insoluble ash	0.184
Sulphated ash	0.176
Moisture ash	0.023
Estimation values for 1g of the sample	

The Summarises various physicochemical characteristics of *Tecoma stans* flower powder. The acid insoluble ash content is found to be higher next to the total ash content in the table. The moisture content is rather very low. These parameters are useful mainly for the purposes of standardization of plant drug. Generally, speaking, higher value of total ash indicates higher organic constituents being present in the study plant. If the acid soluble ash values is higher, indicates more insoluble inorganic compounds be present in the plant parts. A moisture content is a very good indicator of microbial contamination in the plant parts.

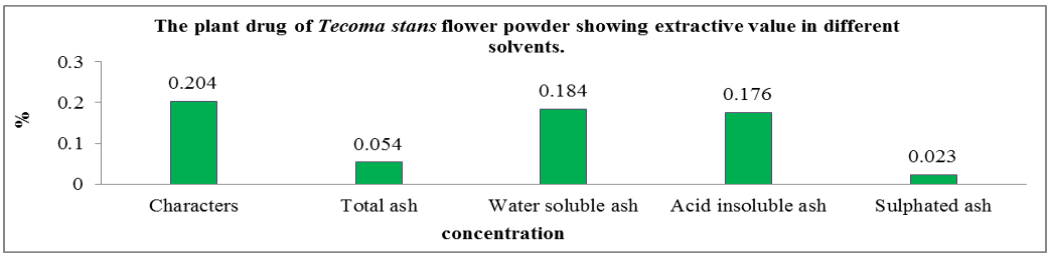

Figure 1: The plant drug of *Tecoma stans* flower powder showing extractive value in different solvents.

Table 4: The plant drug of *Tecoma stans* flower powder.

S.No	Secondary Metabolites	Result (mg/gm) Flower
1.	Total phenol	238.21±14.65
2.	Saponin	41.32±2.05
3.	Alkaloids	80.32±4.66
4.	Flavonoids	189.74±12.44

The different extract distinctly shows the presence of various phyto constituents in the plant, such as alkaloids, flavonoids, phenols, carbohydrates, steriods, terpenoid in Tecoma stans flower powder. The certain phytoconstituents which made the presence in ethanolic solvent also failed to appear in ethyl acetate solvent.

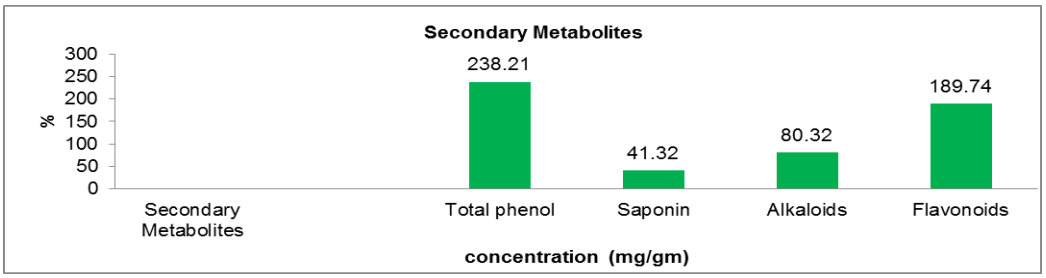

Figure 2: The plant drug of *Tecoma stans* Flower powder.

Values are expressed as Mean ± SD for triplicates

Table 5: Preliminary Phytochemical Analysis of *Tecoma stans* Flower powder.

Phytochemical Constituents	Methanol	Ethanol	Water	Ethyl Acetate
Alkaloids	+	+	+	-
Flavonoid	+	+	+	-
Tannins	+	-	+	-
Phenols	+	+	+	+
Carbohydrate	+	+	+	+
Steriods	+	+	+	+
Saponins	+	-	+	-
Terpenoid	+	+	+	+

(+) indicates Present (-) indicates Absent

Narrates qualitative phytochemical analysis of *Tecoma stans* flower powder in different solvents. A glance at this table, indicates the presence and absence of various phyto constituents of this flower powder.It is noteworthy to mention here that, ethanolic extract of the plant, yielded higher constituents, then other extractive solvent. Therefore, this extract is selected for further study here.

Various organoleptic characteristics of the plant *Tecoma stans* flower powder and the details are shown in Table 1.The preliminary observation on the organoleptic characteristics of the study plant form the base line investigations into any herbal drug study and the details shown here are self-explanatory. These parameters will be useful for standardization purpose of plant drug.

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phytoconstituents which made the presence in ethanolic solvent also failed to appear in ethyl acetate solvent.

Table 6: Estimation of anti-microbial activity of *Tecoma stans* flower. by Agar well diffusion method.

Type	Organism	Control	25 µl	50 µl	75 µl	100 µl
Fungus	*Aspargillus Niger*	-	-	-	1.8 mm	2 mm
	Aspargillus fumigates	-	-	1.2 mm	1.5 mm	3.5 mm
Bacteria	*Klebsiella pneumonia*	-	-	1.1 mm	1.3 mm	1.8 mm
	Pseudomonas aeruginosia	-	-	-	-	1.2 mm
	Bacillus sp	-	-	1 mm	1.5 mm	2 mm

Its Shows the estimation of antimicrobial activity of Tecoma stans by agar well diffusion method. In this antibacterial effect is seen for Klebsiella pneumonia, Pseudomonas aeruginosia, Bacillus sp. Among three bacteria species the flower ethanolic extract show maximum effect at 100µl against Bacillus sp and minimum effect in Pseudomonas aeruginosia. Similarly antifungal effect is seen for two fungal species Aspargillus nigerm and Aspargillus Fumigates.Among these two fungal species Aspargillus Fumigates show maximum antifungal effect.The flower extract has more or less same effect in bacterial species and fungal species.

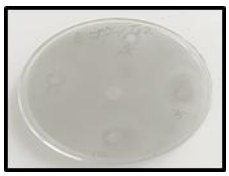

Fig. 3: *Aspargillus.*

Fig. 4: Aspargillus fumigates.

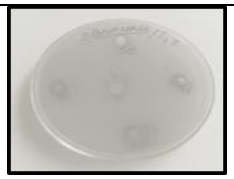

Fig. 5: Klebsiella pneumonia.

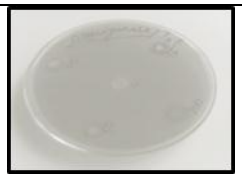

Fig. 6: Pseudomonas aeruginosia.

Table: 7 Antioxidant results (DPPH method) using ethanolic extract of plant drug.

Samples	Concentration (µg/ml)				
	25	50	75	100	125
Tecoma stans ethanolic extract	52.82 ±0.22	54.30 ±0.16	58.26 ±0.23	62.38 ±0.30	69.30 ±0.32
Ascorbic Acid (Standard)	84.2 ±0.14	86.7 ±0.28	88.9 ±0.28	92.1 ±0.14	94.8 ±0.07

The estimation of antimicrobial activity of Tecoma stans by agar well diffusion method. In this antibacterial effect is seen for Klebsiella pneumonia,Pseudomonas aeruginosia, Bacillus sp. Among three bacteria species the flower ethanolic extract show maximum effect at 100µl against Bacillus sp and minimum effect in Pseudomonas aeruginosia. Similarly antifungal effect is seen for two fungal species Aspargillus nigerm and Aspargillus Fumigates.Among these two fungal species Aspargillus Fumigates show maximum antifungal effect.The flower extract has more or less same effect in bacterial species and fungal species.

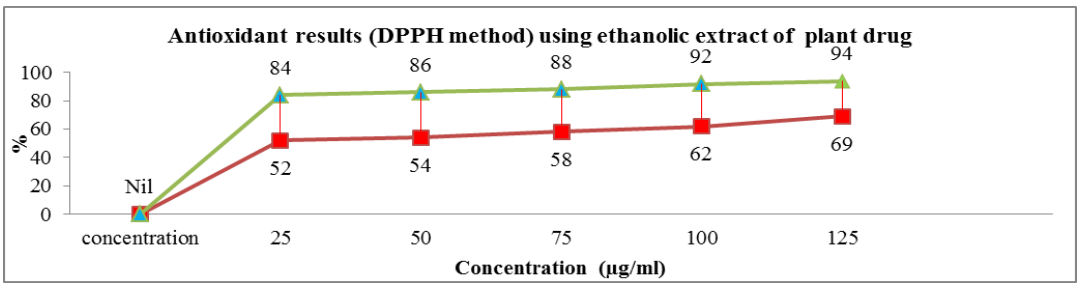

Fig. 7: Antioxidant results (DPPH method) using ethanolic extract of plant drug.

The Summarises of Antioxidant results by using DPPH method. Percentage of scavenging activity at different concentration of plant drug ethanolic extract and standard ascorbic acid are compared. The percentage of scavenging activity even though less than standard it has good scavenging activity at concentration 125 µl (i.e., nearly 69.30 %).

UV spectrum (Fig. 8) shows two peaks which are in band region I ie 289 nm and another at band region II at 208 nm. It confirms the presence of flavonoids.

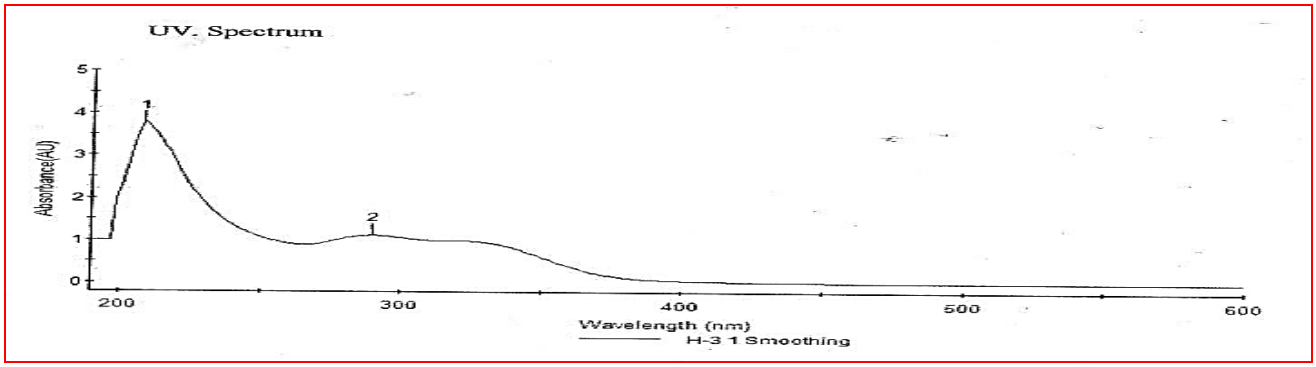

Fig.8: UV spectrum of isolated compound from *Tecoma stans* flower powder.

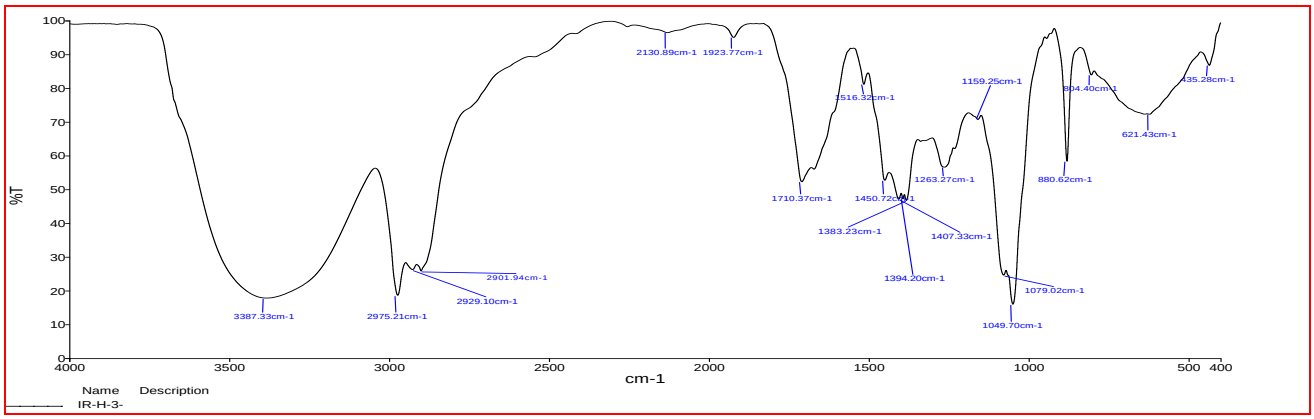

Fig. 9: IR spectrumof isolated compound from *Tecoma stans* flower powder.

The IR spectrum (Fig. 9) shows peaks. The FTIR gave broad peaks at 3387.33cm-1 which indicated the presence of O-H stretching. It showed strong peaks at 2975.21, 2130.89 and 1723.77cm-1 which indicated the presence of alkane, alkyne and aldehyde whereas the peak 1450.72 and 804.40cm-1 suggested the presence of aromatic functional groups present in the separated compound which are related to the flavonoid. Further spectral studies are required to confirm the structure of the flavonoid.

4. CONCLUSION

From these preliminary findings it is concluded the plant drug *Tecoma stans* flower powder posses antimicrobial and anti- oxidant activity. In this plant drug one of the main consitutent is flavoniod which is confirmed by spectrum. However further phyto chemical,bio screening and spectral studies need to confirm the structure of the isolated flavonoid and mechanism of the plant drug.

5. ACKNOWLEDGMENT

Authors are hearty thankful to the authorities and technical staff of AVVM Sri Pushpam college Bharathidasan University for providing facility to carry out the work. I wish to thank my supervisor Dr. R. Manikandan, Msc., Mphil., Ph.D., for his guidance, valuable suggestions and stimulating explanations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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