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To determine the antioxidant mechanism of action, biochemical compounds in *Tecoma stans* (L. Juss. ex Kunth) were analyzed using GC-MS, HPLC, UV-VIS, and FTIR techniques

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ABSTRACT

The alkaloids found in *Tecoma stans* (L.Juss.ex Kunth) are well-known and have been utilized extensively in traditional Chinese medicine. On the other hand, the phenolic compounds have not been sufficiently studied. Thus, the objective of this study was to qualitatively and quantitatively examine the phenolic chemicals found in the plant's roots and leaves. Either methanol or ethanol were used in the extraction processes; however, ethyl acetate and water worked better than methanol and ethanol. Phenolic chemicals were more abundant in the leaves than in the roots. The DPPH technique yielded values for the antioxidant activity of 46.59 µg/ml, 40.51 µg/ml, and 34.89 µg/ml, in that order. The existence of phenolic compounds was confirmed by FTIR and the UV-VIS spectra. Using negative and positive ionization, GC-MS was used to examine the phenolic profile. A total of 15 compounds were identified, and HPLC analysis revealed the existence of 8 compounds that would be useful in pharmaceutical and medical applications.

Key words: *Tecoma stans (L.Juss.ex Kunth), antioxidant, GC MS, HPLC, UV-VIS, FTIR*

Introduction

Tecoma stans, a plant known for its leaves and roots, contains two alkaloids, tecomanine and tecostanine, which have been found in its roots and leaves. These leaves and roots have been used in traditional folk medicine due to their physiologically active compounds, including flavonoids, phenols, and monoterpenes. The plant also has tonic, diuretic, and anti-syphilitic properties. *Tecoma stans* is not harmful and is used to treat diabetes. The root-related antifungal action is primarily found in flavonoid, which has been purified through HPLC and column chromatogram. Flavonoids have been shown to prevent diabetes, heart disease, dementia (Navaneethan *et al*., 2016), and several forms of cancer. Techniques such as GC-MS, HPLC, UV-VIS, and FTIR can provide insight into the use of *Tecoma stans* in traditional medicine (Katta *et al*., 2016).

Materials and Methods

Preparation of plant material

The rhizomes of *Tecoma stans* (Family: Bignoniaceae) were collected, powdered, cleaned, and dried in the shade. The powder was sealed in an airtight polythene container and stored for further analysis.

The prepared herbarium specimen was kept at the Department of Botany and Microbiology of A.V.V.M. Sri Pushpam College, located in Poondi, Thanjavur, Tamil Nadu, India.

Preparation of plant extract

In order to eliminate dust and other debris, the plant samples were meticulously cleaned with water. After that, 3 kg of thoroughly cleaned leaf samples were dried at 25 ºC for seven days in the shade. The samples of leaves and roots were dried, and then they were processed into a powder in a grinder for 30 seconds. Aqueous, methanol, ethanol, ethyl acetate, and water were used as solvents in a variety of Soxhlet extraction techniques to extract the powdered material. After that, the extract was concentrated and stored at 4 °C until the equipment required it once again. After the defatted powder constituents were further extracted using ethanol, concentrated extracts were used for the evaluation.

Qualitative evaluation of phytochemicals

The several kinds of secondary metabolites, including alkaloids, carbohydrates, tannins, sapiens and phenolic compounds, flavonoids, and coumarone, that were discovered in the crude ethanol tuber extract were identified by early phytochemical study using multiple standard tests.

UV and FT-IR Spectroscopic analysis

The extract was examined in both visible and ultraviolet light for proximate analysis. The extracts were prepared for UV and FT-IR spectrophotometer analysis by filtering them using Whatmann No. 1 filter paper and centrifuging them for 10 minutes at 3000 rpm. The sample was diluted to a ratio of 1:10 using the same solvent (Anburaj *et al.,* 2016). The characteristic peaks were located by scanning the extract in the 200-1100 nm wavelength range with a Perkin Elmer spectrophotometer. FTIR analysis was performed using the Perkin Elmer spectrophotometer equipment to determine the characteristic peaks and their functional groups, which varied in size from 400 to 4000 cm-1. Peak values for both FT-IR and UV were recorded. Each analysis was performed twice to ensure the spectrum was substantiated.

HPLC Analysis

The flavonoid fractions were subjected to HPLC analysis. A chromatographic system (Shimadzu Class-VPV6.14SP2, Japan) equipped with an auto sampler with a 20 µl fixed loop and a UV-visible detector was used to analyze the extract using HPLC. The gradient elution of solvents B (methanol) and A (water-acetic acid, 25:1 v/v) had a major effect on the resolution of molecules. Solvent gradients were thus produced using a dual pumping system by varying the ratio of solvent A (water-acetic acid, 25:1, v/v) to solvent B (methanol). Solvent B was then increased to 50% in 4 minutes, and then to 80% at a flow rate of 1.0 milliliters per minute in 10 minutes. A UV detector (Lamp-D2) was used to detect the samples at 280 nm for a duration of 25 minutes. All chromatographic data was recorded and processed using autochrome software.

GC-MS Analysis

The GC-MS analysis was conducted using Shimadzu 2010 Plus. It had the following operational parameters, a gas chromatograph interfaced to a mass spectrometer, and an AOC-20i auto simpler. This column, known as the RTX 5Ms, is 30 meters long, 0.32 mm in diameter, and 0.50 µm thick. In electron impact mode, it functions at 70 eV. The ion source is 200 °C, the injector is 270 °C, and the carrier gas employed is 99.999% helium gas flowing at a steady 1.73 ml/min rate. The oven's settings were as follows: it started at 40 °C (isothermal for 2 minutes), increased by 8 °C/min to 150 °C, increased by 8°C/min to 250°C, and ended with a 20-minute isothermal at 280 °C. At a scan period of 0.5 seconds, mass spectra of fragments ranging in size from 40 to 450 Da were acquired at 70 eV. The GC lasts for a total of 51.25 minutes (Anand *et al.,* 2021). The relative percentage quantity was ascertained by comparing each component's average peak area to the overall areas. 5.2.0 Turbo Mass Ver. remained the mass spectra and chromatogram management program.

DPPH Radical Scavenging Assay

The antioxidant assay of *Tecama stans* extract was conducted using UV spectrophotometry at 517 nm. The extracts were produced at different doses and ascorbic acid was used as a standard. The reaction took 30 minutes at room temperature, with 1 ml of each extract, 3 ml of each solvent, and 0.5 ml of 1.0 mM DPPH in ethanol as a control. A triplicate sample was generated for each analysis, and the absorbance mean was recorded. The DPPH radical scavenging was calculated using the formula

DPPH inhibition % = $[(A_0 - A_1)/A_0] \times 100$,
where A_1 represents the extracts or ascorbic acid's absorbance and A_0 is the control's absorbance. The plant extract's inhibitory concentration (IC-50) was calculated by lowering the starting DPPH concentration by 50%.

Statistical analysis

The values are presented as the mean ± SD (standard deviation) of triplicate measurements.

Results and Discussion

The UV-VIS spectral analyses of the plant extract show the presence of broad peaks at 202.6, 250.1, and 745.3 nm, which are indicative of phenolic compounds. The results of the UV-VIS spectroscopic examination shown in (Figure 1) verify the presence of flavonoids in the plant extract. The UV-visible absorbance spectra of the ethanol extracts were measured between 200 and 1100 nm. Peaks with absorptions of 3.758 and 0.190 at 279.85 and 1062.95 nm, respectively, were seen in the profile. The *Tecama stans* extract spectra show two peaks at wavelengths of 353 nm and 407 nm.

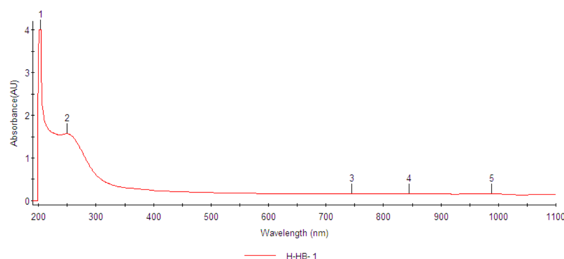

Fig. 1. UV Visible spectrum of *Tecama stans* leave extract

Table 1. UV-Visible spectrum of *Tecama stans* Leaves Extract assigned peak values

S. No.	Wave length (nm)	Peak value (Au)
1	202.6	4
2	250.1	1.571
3	745.3	0.168
4	843.9	0.168
5	988.2	0.159

Fourier transforms infrared spectroscopy

The FT-IR spectra of the plant extract (shown in Figure 2) indicates the presence of functional groups with corresponding absorbance frequencies cm^{-1}. At 3423 cm^{-1}, a very strong broad absorbance peak was found, indicating that phenolic compounds are stretching their O-H bonds. Bands at 3423 cm^{-1}, 2918 cm^{-1}, 2848 cm^{-1}, 2368 cm^{-1}, 1593 cm^{-1}, 1375 cm^{-1}, 1020 cm^{-1}, and 678 cm^{-1} were found in the extract FTIR spectra (Veeravelan *et al.*, 2024).

Amino acids, carboxylic acids, methyls, esters, alcohols, alkyls, alkanes, alkynes, and alkenes are the FTIR results. The absorption at 3349.81 cm^{-1} is the result of the extract's hydroxyl groups being stretched (Table 2). The band at 2927.23 cm^{-1} is the result of symmetrical stretching of saturated (sp3) carbon. The band at 1633.44 cm^{-1} is attributed to the bending mode of absorbed water, as plant extracts are known to have a high affinity for water. The band at 1537.09 cm^{-1} is caused by the C=C stretching and aromatic skeletal mode of the extracts.

The extracts' aromatic skeletal mode is linked to C=C stretching, which is responsible for the band at 1537.09 cm^{-1}. The vibrational absorption band at 1384.66 cm^{-1} was ascribed to the methyl group's trembling. A noticeable band at 1253.97 cm^{-1} and 1054.89 cm^{-1} may be related to C-O stretching. A characteristic band for the aromatic H out of plane bending is located at 599.76 cm^{-1}.

Gas Chromatography-mass Spectroscopy Method (GC-MS)

Gas Chromatography-Mass Spectroscopy Method for the Identification of Bioactive Compounds in Plant Mixture Extract The spice blend extract's 15 components were identified by GC-MS analysis. The Retention Time (RT), Molecular Weight (MW), Molecular Formula (MF), and Concentration (%) of the active principles are used to represent them. Hepatic protective, antioxidant, anti-inflammatory, ant preventive, antihistaminic, ant coronary, asthma-preventive, antitumor (breast, lung, and prostate), and antitumor (nasopharynx) are among the characteristics of biologically active compounds. The modest peaks could be caused by substances existing in little quantities or by massive molecules dissolving.Low retention times are linked to the principal plant compounds that have low polarity (*Heryanto Heryanto et al.,* 2024). The technique known as gas chromatography-mass spectrometry, or GC-MS, combines the features of gas-liquid chromatography with mass spectrometry to detect different materials in a test sample.

HPLC Analysis of *Tecama stans*

The result is proven to include a number of fla-

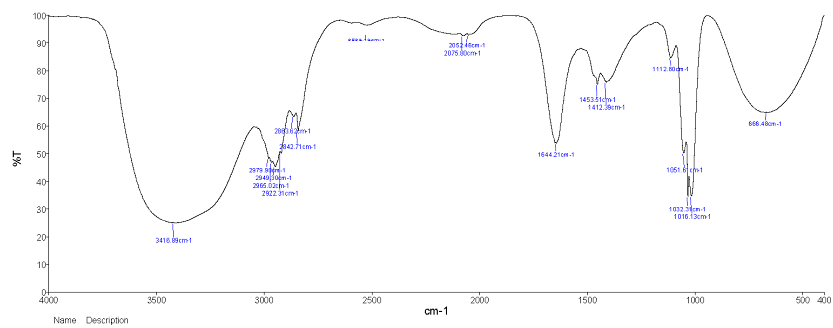

Fig. 2. FTIR spectrum analysis of *Tecama stans*

Table 2. Identification of Functionnal group in sample by FTIR spectrum (shown in Fig. 2).

Peak cm^{-1}	**Bond**	**Functional groups**
3366.11	O–H stretch, H–bonded	Alcohol and phenol
2979.90,2949.30, 2965.02,2922.31	C-H stretch	Alkanes
1644.21	-C=C- stretch	Alkenes
1453.51	C-C stretch (in-ring)	Aromatic
1112.80, 1051.61,1032.31	C-O stretch	Alcohols, carboxylic acids, esters, ethers
666.48	=C-H bend	Alkenes

vonoids, such as quercetin, gallicapigenin, resorcinol, caffeic acid, and caffeic cyanidin-3-O-glucoside (-). The extract from the spice combination contains kaempferol (-) and epicatechin (-). The compound flavonoids is found in many different plant species. It is further divided into isoflavones, anthocyanidins, proanthocyanidins, flavonols, flavones, flavanones, and anthocyanidins, each of which has a distinct component **[18].** The compound flavonoids is found in many different plant species. It can be further divided into isoflavones, anthocyanidins, proanthocyanidins, flavones, and flavones, each of which has a distinct feature. It was found that the best solvent was ethanol. because its concentration of bioactive components was the highest. While flavonoids declined dramatically, several

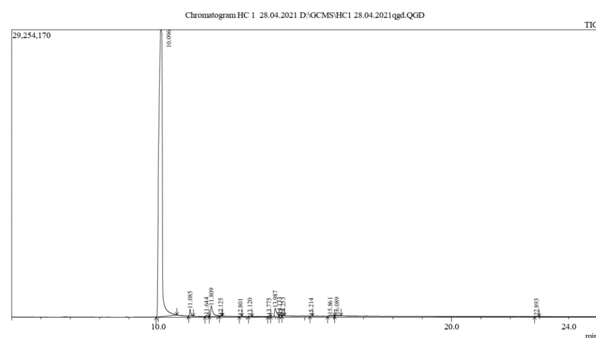

Fig. 3. Gas chromatogram and mass spectrum of sample

flavonoids showed a high proportion in Hawraman hawthorn (18.24%, 13.72%, and 11.98%, respectively), such as quercetin and apigenin.In Qaradax hawthorn, on the other hand, certain flavonoids, such as nonacosan-10-01, kaempferol, and Quercetin, dominated with the following ratios (18.10%, 16.59%, and 15.51%, respectively).

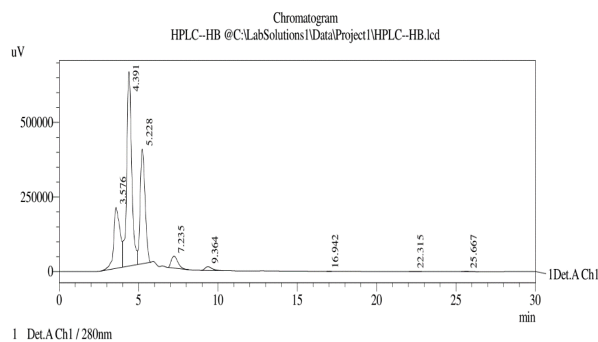

Fig. 4. HPLC Analysis of *Tecama stans*

DPPH radical scavenging activity of mixed spices extract

The evaluation of the ethanol extract from leaves and roots' antioxidant qualities is displayed in Figure 5. A popular technique for assessing the antioxidant capabilities of plant extracts is the DPPH assay. Figure 5 illustrates the comparison between ascorbic

Table 3. Identification of bioactive compounds in sample by GC- MS analysis

Peak	R. Time	Area %	Height %	Molecular Formula	Molecular Weight	Name of the compounds
1	10.096	94.09	89.09	$C_{12}H_{14}O_4$	222	Diethyl Phthalate
2	11.085	0.72	2.21	$C_{12}H_{14}O_4$	222	1,2-Benzenedicarboxylic acid, diethyl ester
3	11.644	0.15	0.46	$C_{12}H_{14}O_4$	222	2-(Isobutoxycarbonyl)benzoic acid
4	11.809	2.38	3.4	$C_{14}H_{28}O_2$	228	Tetradecanoic acid
5	12.125	0.02	0.08	$C_{18}H_{36}O_2$	284	Hexadecanoic acid, ethyl ester
6	12.801	0.04	0.13	$C_{15}H_{21}N_3O_2$	275	Benzoic acid, 4-(1-azepinyl)azo-, ethyl ester
7	13.12	0.07	0.19	$C_{26}H_{42}O_4$	418	Bis-(3,5,5-trimethylhexyl) phthalate
8	13.775	0.08	0.27	$C_{17}H_{24}O_3$	276	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-dien
9	13.987	1.5	2.45	$C_{18}H_{36}O_2$	284	Octadecanoic acid
10	14.133	0.18	0.4	$C_{20}H_{26}O_4$	330	Phthalic acid, di-(1-hexen-5-yl) ester
11	14.253	0.09	0.24	$C_{14}H_{28}O_2$	228	Dodecanoic acid, ethyl ester
12	15.214	0.03	0.1	$C_{36}H_{75}$	587	Phosphonic acid, dioctadecyl ester
13	15.861	0.34	0.35	$C_{18}H_{32}O_2$	280	9,12-Octadecadienoic acid (Z,Z)-
14	16.089	0.24	0.32	$C_{18}H_{34}O_2$	282	9-Octadecenoic acid (Z)-
15	22.893	0.07	0.12	$C_9H_{20}O$	144	1-Octanol, 2-methyl- $$ 2-Methyl-1-octanol

Table 4. Biological activity of phyto-components identified in sample by GC-MS
**Source: Dr. Duke's phytochemical and ethno botanical databases [Online database].

Peak	R. Time	Name of the compounds	Biological activity**
1	10.096	Diethyl Phthalate	Antimicrobial, Acetyl cholinesterase and Neurotoxic activity
2	11.809	Tetradecanoic acid	Antioxidant, Cancer preventive Nematicide, Hypocholesterolemic and Lubricant
3	12.125	Hexadecanoic acid, ethyl ester	Antioxidant, Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic
4	13.775	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-die	Antimicrobial activity
5	13.987	Octadecanoic acid	Antioxidant, anti-inflammatory and Antimicrobial activity
6	14.133	Phthalic acid, di-(1-hexen-5-yl) ester	Antimicrobial and Antifouling activity
7	14.253	Dodecanoic acid, ethyl ester	Antibacterial, Antiviral, Antioxidant, Candidicide and Hypercholesterolemic activity
8	15.861	9,12-Octadecadienoic acid	Hypocholesterolemic, Nematicide, Antiarthritic, Hepatoprotective, Antiandrogenic, Hypocholesterolemic 5-Alpha reductaseinhibitor, Antihistaminic, Anticoronary, Insectifuge, Antieczemic and Antiacne
9	16.089	9-Octadecenoic acid	Anti-inflammatory, antiandrogenic cancer preventive, dermatitigenichypocholesterolemic, anemiagenic and insectifuge

acid and Tecoma Stans leaves' ability to scavenge DPPH radicals in this study. The ascorbic acid, roots extract, and leaf extract had IC-50 values of 34.89 µg/ml, 40.51 µg/ml, and 46.59 µg/ml, respectively. The plant extract inhibited DPPH activity in a dose-dependent manner. The content of L-ascorbic acid directly affects its capacity to scavenge DPPH radicals. Out of the leaf and root extracts, the root extract showed the most promise and was almost as active as the standard.

Conclusion

Respiratory infections can be inhibited by minerals and phytochemicals. Phenolic compounds and their derivatives at visible spectroscopy peaks at 202.6, 250.1, and 745.3 nm are present in the spice mixture's UV extract. The FTIR data verified the existence of carboxylic acids, esters, ethers, phenols 1112.80, 1051.61, and 1032.31 C-O stretch alcohols, 3366.11 O-H stretch, and H-bonded alcohol. GC-MS was used to determine the compounds in the

Table 5. HPLC analysis of *Tecama stans**(Baram Ahmed [2]Paranthaman[3]

Peak#	Ret. Time	Area	Height	Area %	Height %	Compounds identified by literature*
1	3.576	5646975	204074	18.819	15.79	Quercetin
2	4.391	14458315	651188	48.184	50.385	Gallic Acid
3	5.228	14458315	384109	27.78	29.72	Apigenin
4	7.235	14458315	39956	3.908	3.092	Resorcinol
5	9.364	369061	12170	1.23	0.942	Caffeic Acid
6	16.942	137	14	0	0.001	Cyanidin -3-O-glucoside
7	22.315	2508	98	0.008	0.008	(-)-Epicatechin
8	25.667	20927	813	0.07	0.063	Kaempferol
Total		30006529	1292422	100	100	

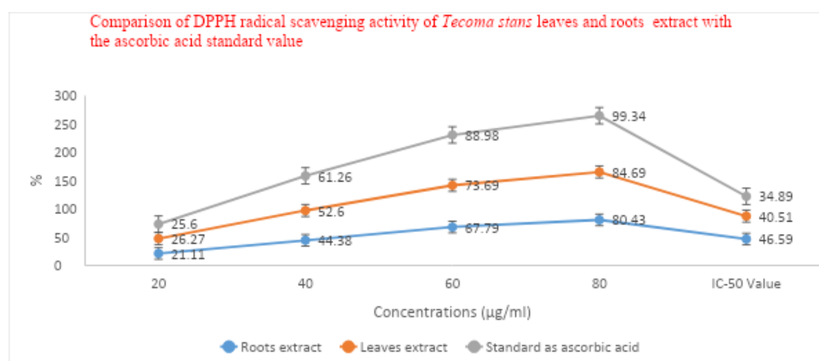

Fig. 5. DPPH radical scavenging activity of mixed spices extract

sample's biological activity. Fifteen compounds exhibited biological activity; most of them had anticancer effects in lung cancer together with antioxidant, anti-inflammatory, and especially anti-cold qualities. Nematicide is an antioxidant that also functions as a lubricant and decreases cholesterol.The flavonoids 46.59 g/ml, 40.51 g/ml, and 34.89 g/ml were independently identified by HPLC *Tecama stans*re gular goods remove and ascorbic damage. Cyanidin -3-O-glucoside (-)-Epicatechin, Apigenin, Resorcinol, Caffeic Acid, Quercetin, and Kaempferol. Because of this, the ethanolic extract of the spice mixture extract contains a high concentration of bioactive compounds, particularly flavonoids, which have antioxidant and anti-inflammatory qualities.

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Ethical Clearance: Not required

Declaration of Competing Interest

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