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Phytochemical screening and characterization of the bioactive compounds from the leaves of *Hyptis suaveolens* and *Spathodea campanulata*

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ABSTRACT

Plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from plant sources are easily available, less expensive, safe, and efficient and rarely have side effects. Preliminary screening of phytochemicals is a valuable step in the detection of bioactive principles present in medicinal plants and may lead to novel environmentally friendly bioherbicides and drug discovery. In the present study, principal phytoconstituents from five solvent extracts of Hyptis suaveolens and Spathodea campanulata leaves were screened by using standard methods and resulted in the detection of the presence of tannins, flavonoids, phenolics, terpenoids, steroids, alkaloids and simple sugars. These phytocompounds were found higher in methanolic, aqueous and acetone extracts of both plants and hence they were characterized using UV-VIS and FT-IR to produce the spectrum profile. The crude extracts were scanned in the wavelength ranging from 200-800 nm and the characteristic peaks were detected. FTIR method was performed in mid infrared region 4000-400 cm^{-1} which was used to detect the characteristic peak values and their functional groups. The maximum compounds were found in the methanolic extract of H. suaveolens and the aqueous extract of S.campanulata with the presence of primary amines, alkanes, carboxylic acid, aromatics, alcohols, ketones and phenols. Our findings supported the evidence that these tested plants contain medicinally important bioactive compounds and it justifies their use in the traditional medicines for the treatment of various diseases.

Key words: Phytochemical Screening, UV-VIS Spectrophotometer, FT-IR, *Hyptis suaveolens*, *Spathodea campanulata*.

INTRODUCTION

Medicinal plants are acknowledged as the richest bio-resources and human use of plants as medicinal agents predates recorded history. Ethnomedical plant usage have been extensively utilized in the development of formularies and pharmacopoeias, implementing a major focus in global health care, as well as contributing substantially to the drug development process [1]. As estimated by the WHO (World Health Organization), 80% of the population of some Asian and African countries presently use herbal medicines for some aspect of primary health care [2]. The research on medicinal plants include much more than the discovery of new drugs and this field has been elaborating to include diverse subjects as negotiation of power based on medicinal plant knowledge [3]. Plants produce several secondary metabolite compounds including flavonoids, saponins, steroids, alkaloids, glycosides, glucosinolates and terpenoids to protect themselves from the continuous attack of naturally occurring pathogens and environmental stresses [4]. The analysis of these constituents would help in determining various biological activities of plants. Natural products, either as pure compounds or as standardized plant extracts, provides boundless opportunities for the formulation of novel drug [5]. The secondary metabolites from plants vary in their structural arrangements and properties [6]. A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials [7]. The main advantage of using medicinal plants products as a source of alternative drugs is that they does not produce side effects when compare with synthetic drugs [8]. Natural

products from microbial sources have been the primary source of antibiotics. But with the increasing acceptance of herbal medicine as an alternative form of health care and also with the uprising incidences of multiple resistant organisms, the screening of medicinal plants for their active compounds has become very significant [9,10]. Plants that are endowed with molecules such as vitamins, terpenoids, phenolic acids, tannins, flavonoids, quinones, coumarins, alkaloids and other metabolites are responsible for the antioxidant and free radical scavenging activity [11].

It has been shown that *in-vitro* screening methods could provide the required preliminary observations to select a crude plant extracts with potentially useful properties for further chemical and pharmacological investigations [12]. However, simple, cost-effective and rapid tests are essential for the detection of phytocomponents. Spectroscopic methods such as UV-Vis, FT-IR can be used in this sense as same as conventional methods [13,14]. The Fourier Transform Infrared (FTIR) spectroscopy is an established time-saving method to characterize and identify functional groups [15]. FTIR allows the analysis of compositional and structural information in plants that can be performed both on pure compounds and complex mixtures, without separation into individual components [16]. Ultraviolet-visible (UV-VIS) spectrophotometry uses the light in the visible ranges or its adjacent ranges and the colour of the chemicals involved directly affects the absorption in the visible ranges. Molecules undergo electronic transitions in these ranges of the electromagnetic spectrum [17].

Hyptis suaveolens commonly known as "Wilayati tulsi" belongs to the family Lamiaceae and is an ethnobotanically important medicinal plant. Almost all parts of this plant are being used in traditional medicine to treat various diseases. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities [18]. *H. suaveolens* was targeted on the basis of folkloric uses which suggest its toxicity to microbes, coupled with its importance as food to humans. The leaves of *H. suaveolens* have been utilized as a stimulant, carminative, galactogogue and as a cure for parasitic cutaneous diseases. Crude leaf extract was also used as a relief to colic, stomach ache and was reported to have antimicrobial, antibacterial, antispasmodic, analgesic, anti-inflammatory effects [19-21]. *Spathodea campanulata P. Beauv* is a flowering plant belonging to the Bignoniaceae family. It is commonly known as the Fountain Tree, African tulip tree, Flame-of-the-forest, Rudra Palash, Pichkari or Nandi Flam [22]. The leaves are used against kidney diseases, urethral inflammations and as an antidote against animal poisons. *In-vitro* antimalarial activity against *Plasmodium falciparum* and antibacterial activity of bovine mastitis causing *S.aureus* were evaluated using leaf extracts of *S.campanulata* [23]. *In vitro* antibacterial activity of leaf extracts of this plant against standard strains were also evaluated [24]. The ethanol leaf extract of *S.campanulata* was investigated for its analgesic and anti-inflammatory potentials [25].

With this knowledge, the present investigation was carried out to screen the phytoconstituents present as well as to produce the UV-VIS and FT-IR spectrum profile of the different extracts from the leaves of *Hyptis suaveolens* and *Spathodea campanulata.*

EXPERIMENTAL SECTION

Chemicals

All the reagents used in this study were of analytical grade and were purchased from Sigma–Aldrich Chemical Pvt. Ltd.

Procurement and Processing of Plant Samples

The leaves of *Hyptis suaveolens* and *Spathodea campanulata* were collected from the local areas in and around Chennai and the identification was carried out by a botanist from Central Siddha Research Institute, Chennai, Tamil Nadu. The collected leaves of *H. suaveolens* and *S. campanulata* were washed thoroughly 2-3 times in running tap water followed by sterile distilled water and were dried in hot air oven at 60 ºC overnight. The dried leaves were grounded well with mortar and pestle and these powdered samples were then stored in air tight bottles for further use.

Preparation of Plant Extracts

5g each from the dried powdered samples (*H. suaveolens* and *S. campanulata* leaves) were extracted with 100ml of the solvents such as Water, Methanol, Chloroform, Acetone and Petroleum Ether (50mg/ml) with gentle stirring for 48 hrs. After incubation, the solution was filtered through Whatmann No.1 filter paper and the filtrate were collected (crude extracts). It was then transferred to glass vials and kept at 4°C before use.

Qualitative Phytochemical Analysis

Phytochemical analysis were carried out on the various extracts of *H. suaveolens* and *S. campanulata* leaves using standard procedures to identify the phytochemicals namely, Alkaloids, Simple sugar, Tannins, Steroids, Terpenoids,

Flavonoids, Anthraquinones and Phenols following the methodology of Sofowora, Harborne, Trease and Evans [26-28].

UV-VIS and FT-IR Spectroscopic Analysis

For UV-VIS and FT-IR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whatmann No.1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 200-800 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FT-IR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FT-IR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

RESULTS AND DISCUSSION

Qualitative Phytochemical Analysis

The preliminary phytochemical screening carried out on the five different extracts from the leaves of *H. suaveolens* and *S. campanulata* revealed the presence of various bioactive secondary metabolites that were confirmed from their chemical colour reaction tests as shown in Fig 1 and Fig 2. The results from the phytochemical analysis of *H. suaveolens* leaves extracts showed the presence of alkaloids, simple sugar, tannins, steroids, terpenoids, flavonoids, anthraquinones and phenols in aqueous extract where as the compound anthraquinones was absent in methanolic and acetone extracts as shown in Table 1. By analysing the phytochemical screening results of *S. campanulata* leaves extracts, the methanolic extract showed the presence of alkaloids, simple sugar, tannins, steroids, terpenoids, flavonoids, anthraquinones and phenols, where as alkaloids were absent in aqueous and acetone extracts as shown in Table 2. Only a few of the phytochemicals were found in chloroform and petroleum ether extracts indicating its weak extraction nature of bioactive components from both *H. suaveolens* and *S. campanulata* leaves.

Table 1: Preliminary phytochemical screening of secondary metabolites from *H. suaveolens* extracts

Phytochemical Tests		Solvents				
		Petroleum Ether	Methanol	Chloroform	Water	Acetone
1.	Alkaloids	-	+	-	+	+
2.	Simple Sugars	-	+++	-	+++	+
3.	Tannins	-	+++	+	+	+++
4.	Steroids	+	+++	+++	+	+++
5.	Terpenoids	+	+++	+++	+	+++
6.	Flavonoids	-	+++	-	+++	+++
7.	Anthraquinones	-	-	+++	+++	-
8.	Phenols	-	+++	+	+++	+++

(+++) = Present in high levels; (+) = Present in low levels; (-) = Absence

Table 2: Preliminary phytochemical screening of secondary metabolites from *S. campanulata* extracts

Phytochemical Tests		Solvents				
		Petroleum Ether	Methanol	Chloroform	Water	Acetone
1.	Alkaloids	-	+	-	-	-
2.	Simple Sugars	-	+++	+	+++	+++
3.	Tannins	-	+++	+	+++	+++
4.	Steroids	-	+++	+++	+++	+++
5.	Terpenoids	-	+	+++	+	+++
6.	Flavonoids	-	+++	-	+++	+++
7.	Anthraquinones	-	+	-	+++	+++
8.	Phenols	-	+++	+	+++	+++

(+++) = Present in high levels; (+) = Present in low levels; (-) = Absence

UV-VIS and FT-IR Spectroscopic Analysis

From the analysis of the results obtained in phytochemical screening, it was found that the aqueous, methanolic and acetone extracts of *H. suaveolens* and *S. campanulata* consisted majority of the phytoconstituents and hence these extracts were subjected for the UV-VIS and FT-IR spectroscopic analysis.

The qualitative UV-VIS profile of *H. suaveolens* was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The UV-VIS profile of methanol extract showed the peaks at 288, 324, 347 and 546 nm with the absorption 2.831, 2.481, 2.363 and 0.642 respectively. The aqueous extract profile showed major peaks at 369, 414, 487 and 539 nm with the absorption 2.283, 1.921, 1.593 and 0.516 respectively. The UV-

VIS profile of acetone extract showed the peaks at 317, 328, 336 and 529 nm with the absorption of 2.523, 2.475, 2.378 and 0.488 respectively as shown in Fig 3. The qualitative UV-VIS profile of *S. campanulata* was taken at the wavelength of 200 nm to 800 nm due to the sharpness of the peaks and proper baseline. The UV-VIS profile of methanol extract showed the peaks at 369, 414, 451, 489 and 539 nm with the absorption of 2.275, 1.932, 1.747, 1.423 and 0.545 respectively. The aqueous extract profile of *S. campanulata* showed major peaks at 292, 323, 347 and 545 nm with the absorption 2.631, 2.421, 2.359 and 0.664 respectively. The UV-VIS profile of acetone extract showed major peaks at 369, 412, 460 and 487 nm with the absorption of 2.287, 1.972, 1.745 and 1.596 respectively as shown in Fig 4.

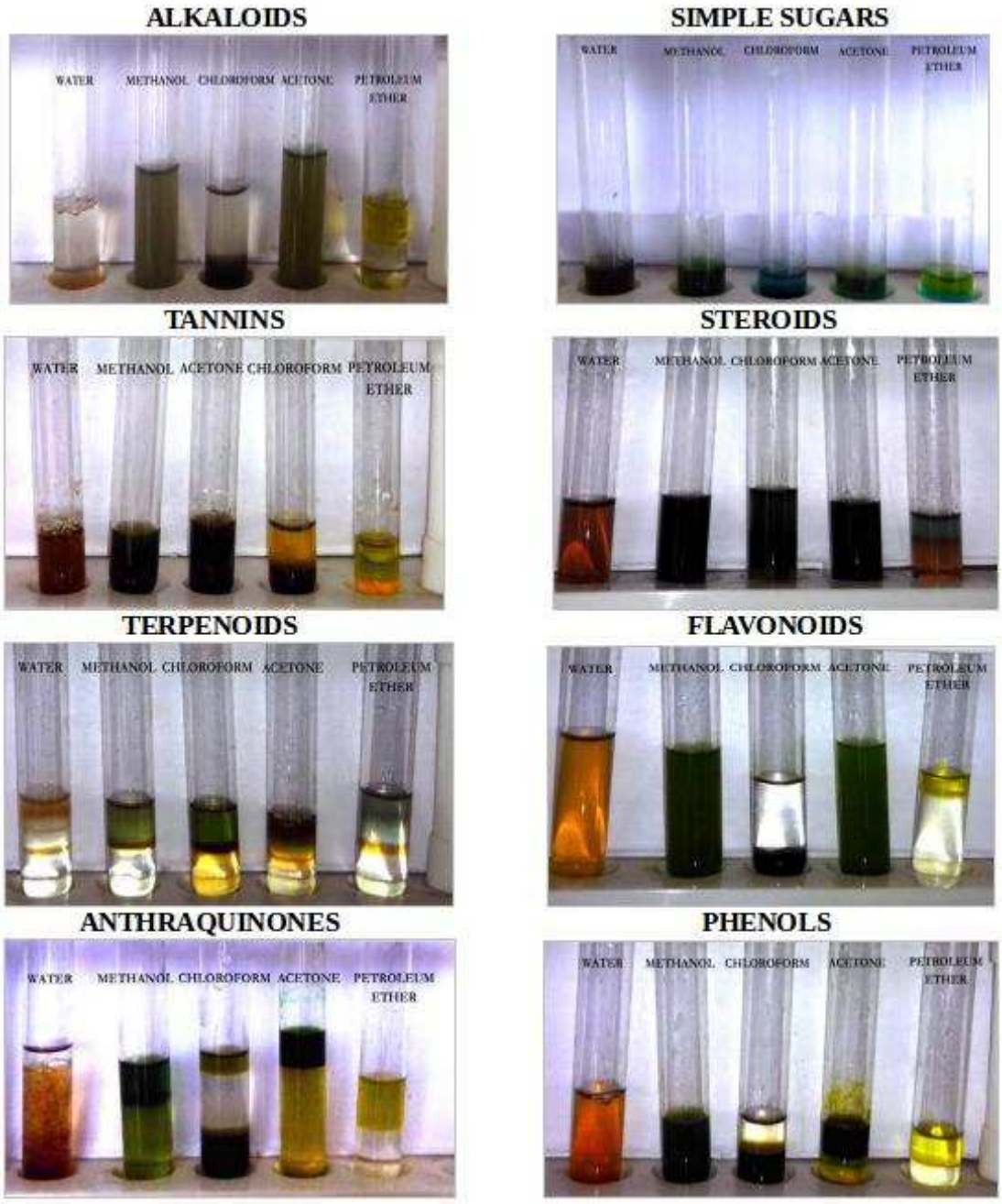

Fig 1: shows the various chemical colour reaction tests for the different extracts from *H. suaveolens* leaves

The FT-IR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The extracts of *H. suaveolens* and *S. campanulata* was passed into the FT-IR and the functional groups of the components were separated based on its peak ratio to obtain the FT-IR spectrums for each solvent as shown in Fig 5. For the plant *H. suaveolens*, the results from the FT-IR analysis of its methanolic extract confirmed the presence of Primary Amine, Bromoalkanes, Chloroaklanes, Carboxylic Acid, Alkane,

Aromatics, Alcohols and Ketone compounds as shown in Table 3. The FT-IR analysis of aqueous extract confirmed the presence of Ammonium Ions, Methylene, Methyl, Aromatic, Alcohols, Phenols, Alkynes and Primary Amine compounds as shown in Table 4. The FT-IR analysis of acetone extract confirmed the presence of Acid Chloride, Methylene, Aliphatic Compound, Methyl, Aromatics, Alcohols, Ketones, Primary Amines and Alkene compounds as shown in Table 5. For the plant *S. campanulata*, the results from the FT-IR analysis of its methanolic extract confirmed the presence of Alcohols, Carboxylic Acid, Aliphatic Compounds, Methyl, Alkenes, Methylene, Primary Amines and Alkane compounds as shown in Table 6. The FT-IR analysis of aqueous extract confirmed the presence of Alkenes, Bromoalkanes, Chloroaklanes, Carboxylic Acid, Aromatic, Alcohols, Primary Amines and Phosphine compounds as shown in Table 7. The FT-IR analysis of *S. campanulata* acetone extract confirmed the presence of Esters, Aliphatic Compounds, Aromatic, Alkenes, Carboxylic Acid, Bromoalkanes, Alcohols and Methylene compounds as shown in Table 8.

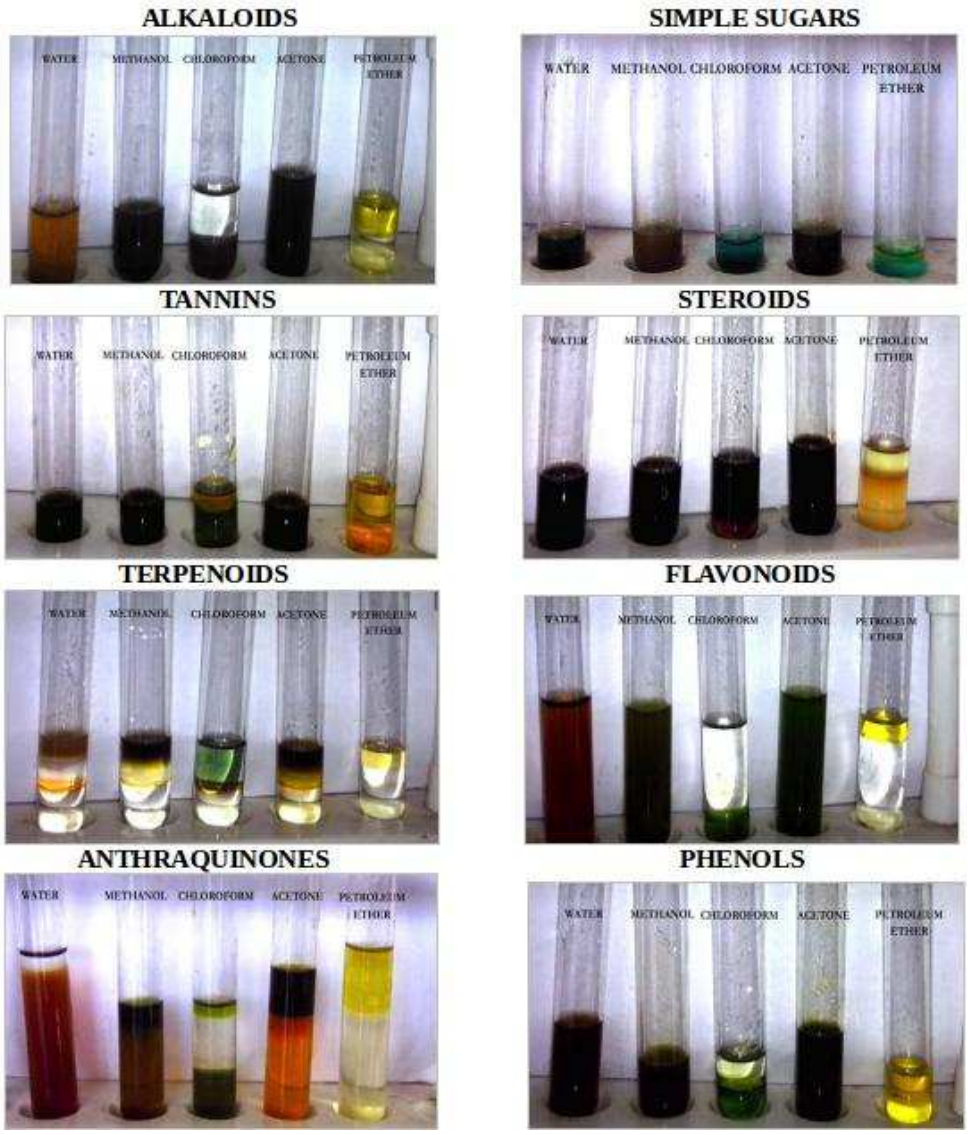

Fig 2: shows the various chemical colour reaction tests for the different extracts from *S. campanulata* leaves

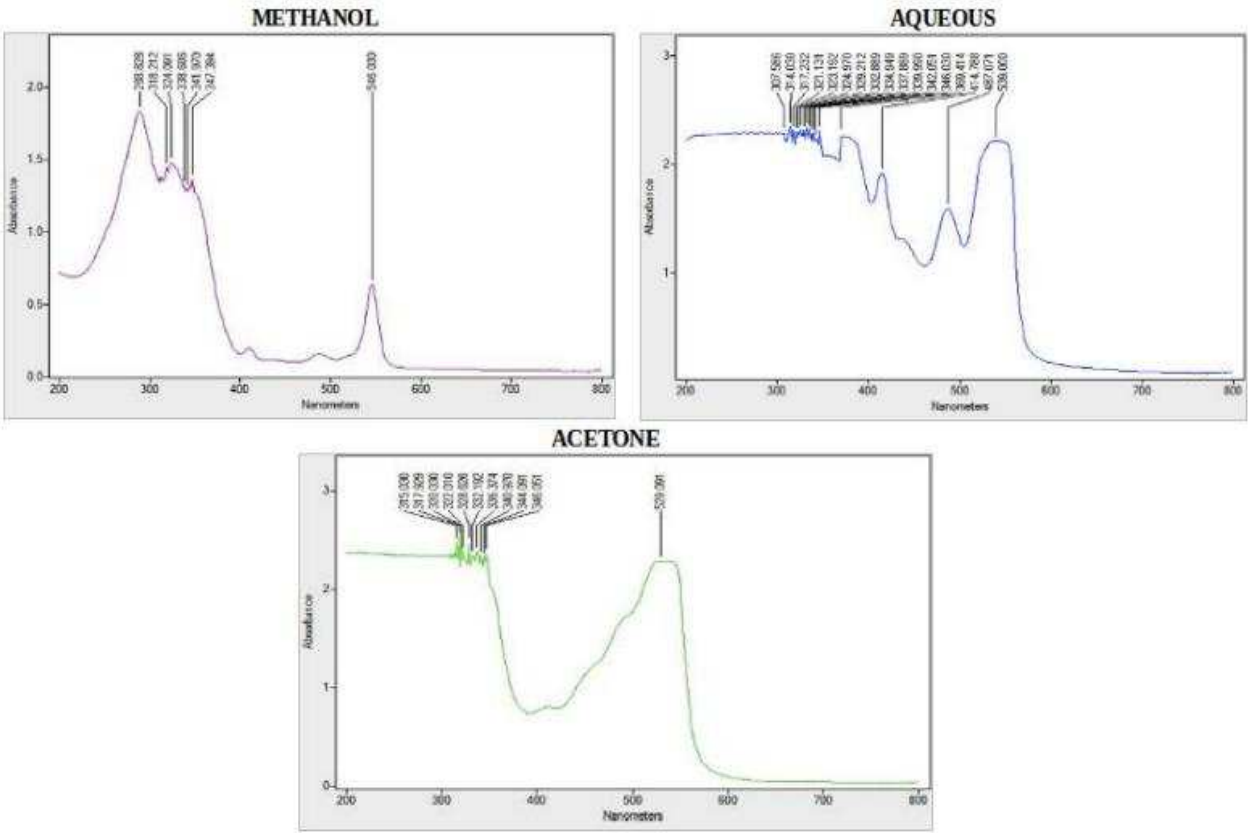

Fig 3: UV-VIS spectrum of *H. suaveolens* methanolic, aqueous and acetone extracts

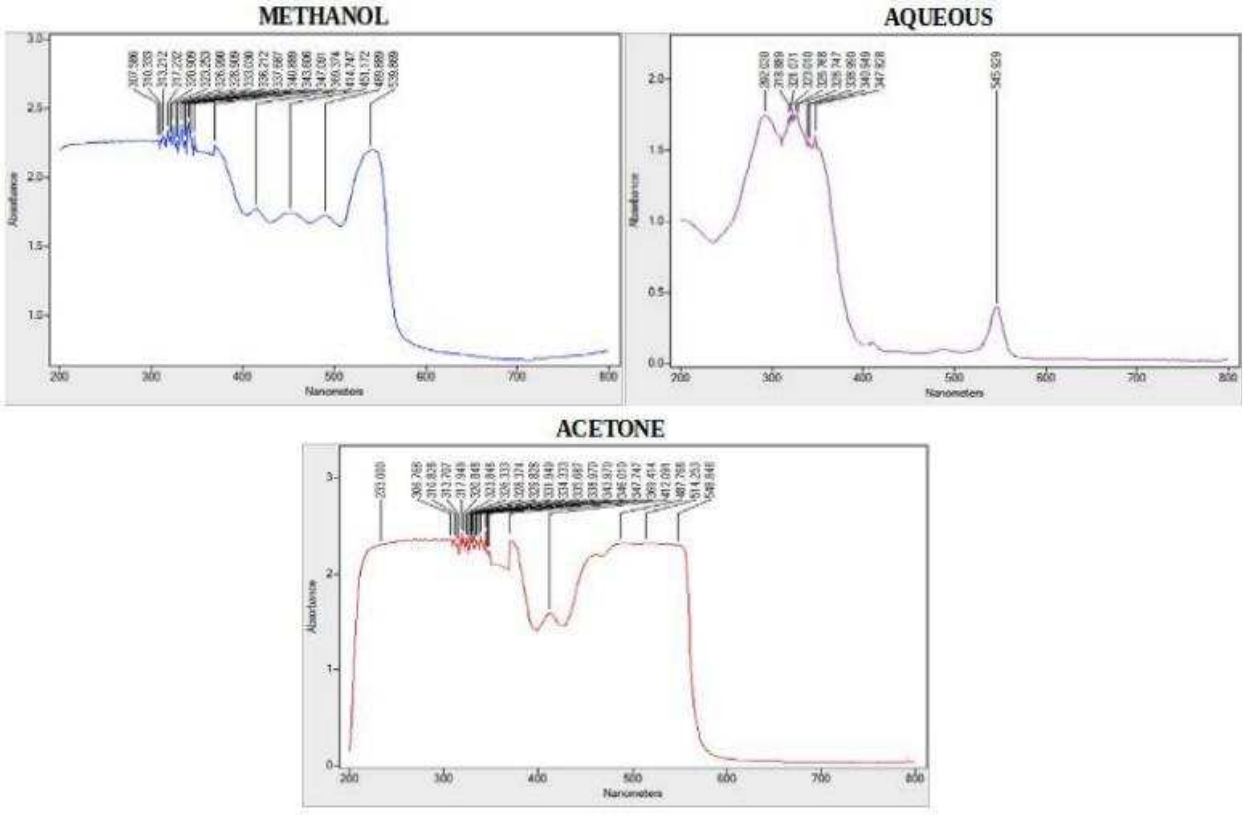

Fig 4: UV-VIS spectrum of *S. campanulata* methanolic, aqueous and acetone extracts

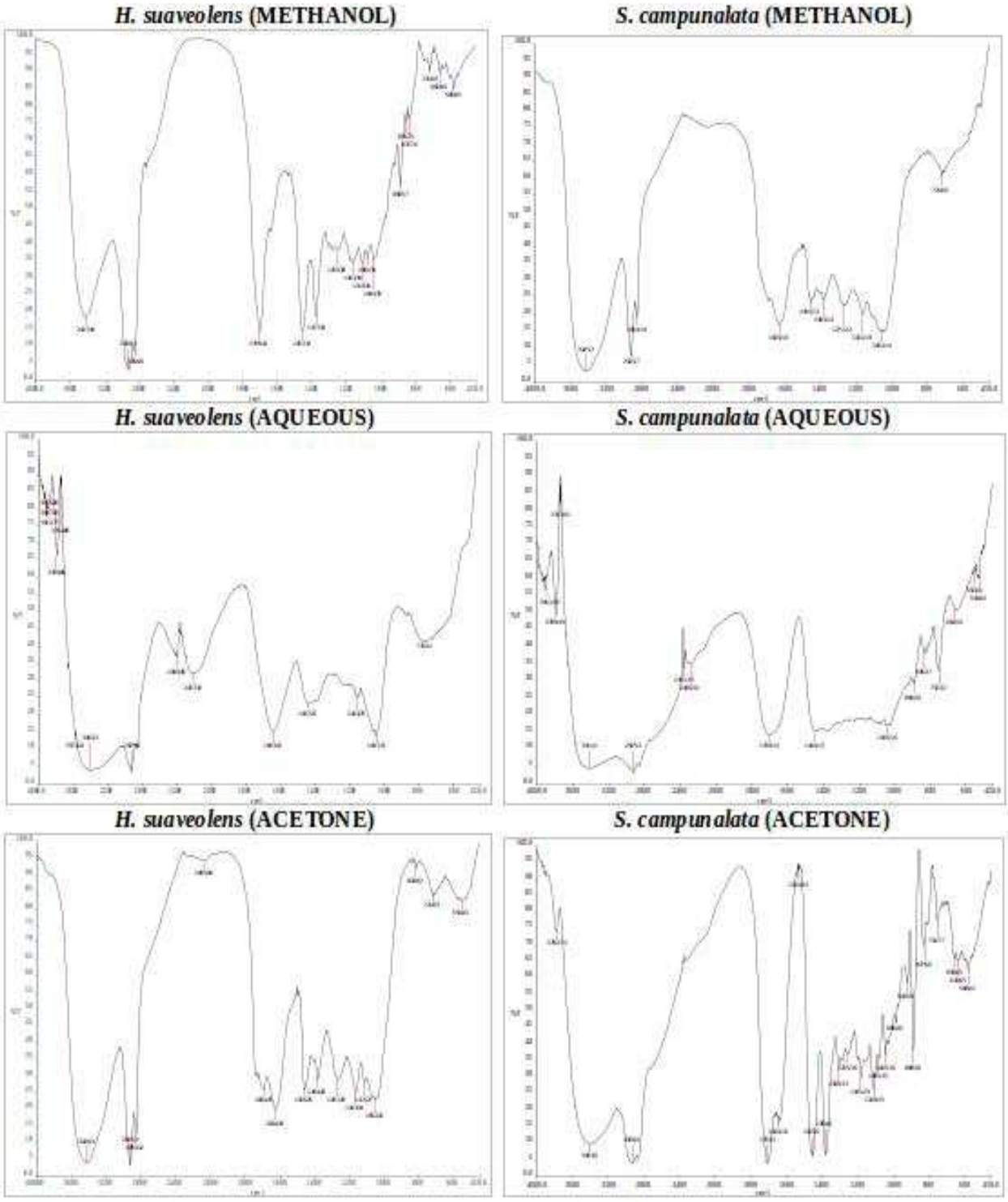

Fig 5: FT-IR Spectrum analysis of methanolic, aqueous and acetone extracts from *H. suaveolens* and *S. campanulata*

Table 3: FT-IR Peak Values of Methanol Extract from *Hyptis suaveolens*

Absorption Peaks (cm^{-1})	Molecular Motion (Bond)	Functional Groups
580	C-Br Stretch	Bromoalkanes (M-S)
662	C-Cl Stretch	Chloroaklanes (W-M)
721	CH_2 Bend	Alkanes (M)
837	C-H Bend (Para)	Aromatics (S)
855	C-H Bend (Para)	Aromatics (S)
890	C-H Bend (Disubstituted)	Alkenes (S)
1044	C-O Stretch	Alcohols (S)(B)
1081	P-H Bend	Phosphines (M)
1110	C-C Stretch	Ketones (S)
1163	C-O Stretch	Alcohols (S)
1255	C-O Stretch	Carboxylic Acid (S)
1377	CH_3 Bend	Alkanes (M)
1457	C-H Bend	Methyl (M-S)
1706	C=O Stretch	Carboxylic Acid (S)
2856	C-H Stretch	Alkanes (S)
2926	C-H Stretch	Methylene (M-S)
3427	N-H Stretch	Primary Amines (S)

M – Medium, S – Strong, W – Weak, B – Broad

Table 4: FT-IR Peak Values of Aqueous Extract from *Hyptis suaveolens*

Absorption Peaks (cm^{-1})	Molecular Motion (Bond)	Functional Groups
770	C-H Bend (Meta)	Aromatics (S)
1047	C-O Stretch	Alcohols (S)(B)
1163	C-O Stretch	Alcohols (S)
1437	C-H Bend	Methyl (M-S)
1643	C=C Stretch (Isolated)	Alkenes (M)
2207	C≡C (Disubstituted)	Alkynes (W)
2400	N-H Stretch	Ammonium Ions (B)
2925	C-H Stretch	Methylene (M-S)
3402	N-H Stretch	Primary Amines (S)
3573	O-H Stretch	Alcohols, Phenols (S)(B)
3744	-	Unknown
3789	-	Unknown

M – Medium, S – Strong, W – Weak, B – Broad

Table 5: FT-IR Peak Values of Acetone Extract from *Hyptis suaveolens*

Absorption Peaks (cm^{-1})	Molecular Motion (Bond)	Functional Groups
554	C-Cl Stretch	Acid Chloride (M-S)
721	CH_2 Bend	Alkanes (M)
820	C-H Bend (Para)	Aromatics (S)
1052	C-O Stretch	Alcohol (S)(B)
1115	C-C Stretch	Ketones (S)
1163	C-O Stretch	Alcohol (S)
1267	C-O Stretch	Carboxylic Acid (S)
1384	N-O Stretch	Aliphatic Compound (W)
1456	C-H Bend	Methyl (M-S)
1630	C=C Stretch (Isolated)	Alkenes (M)
1694	R_2C=N-R Stretch	Imines (S)
2065	N=C=S Stretch	Isothiocyanates (M)
2854	C-H Stretch	Alkanes (S)
2925	C-H Stretch	Methylene (M-S)
3426	N-H Stretch	Primary Amines (S)

M – Medium, S – Strong, W – Weak, B – Broad

Table 6: FT-IR Peak Values of Methanol Extract from *Spathodea campanulata*

Absorption Peaks (cm^{-1})	Molecular Motion (Bond)	Functional Groups
721	CH_2 Bend	Alkanes (M)
1052	C-O Stretch	Alcohols (S)(B)
1162	C-O Stretch	Alcohols (S)
1271	C-O Stretch	Carboxylic Acid (S)
1383	N-O Stretch	Aliphatic Compounds (W)
1455	C-H Bend	Methyl (M-S)
1629	C=C Stretch (Conjugated)	Alkenes (S)
2854	C-H Stretch	Alkanes (S)
2925	C-H Stretch	Methylene (M-S)
3425	N-H Stretch	Primary Amines (S)

M – Medium, S – Strong, W – Weak, B – Broad

Table 7: FT-IR Peak Values of Aqueous Extract from *Spathodea campanulata*

Absorption Peaks (cm^{-1})	Molecular Motion (Bond)	Functional Groups
529	C-Br Stretch	Bromoalkanes (M-S)
552	C-Br Stretch	Bromoalkanes (M-S)
664	C-Cl Stretch	Chloroaklanes (W-M)
752	C-H Bend (Ortho)	Aromatics (S)
836	C-H Bend (Trisubstituted)	Alkenes (S-M)
890	C-H Bend (Disubstituted)	Alkenes (S)
1045	C-O Stretch	Alcohols (S)(B)
1444	O-H Bend	Carboxylic Acid (M)
1700	C=O Stretch	Carboxylic Acid (S)
2286	P-H Stretch	Phosphines (S)
2351	P-H Stretch	Phosphines (S)
2925	C-H Stretch	Methylene (M-S)
3411	N-H Stretch	Primary Amines (S)

M – Medium, S – Strong, W – Weak, B – Broad

Table 8: FT-IR Peak Values of Acetone Extract from *Spathodea campanulata*

Absorption Peaks (cm^{-1})	Molecular Motion (Bond)	Functional Groups
580	C-Br Stretch	Bromoalkanes (M-S)
634	C-Cl Stretch	Chloroaklanes (W-M)
659	C-Cl Stretch	Chloroaklanes (W-M)
754	C-H Bend (Ortho)	Aromatics (S)
827	C-H Bend (Para)	Aromatics (S)
889	C-H Bend (Disubstituted)	Alkenes (S)
924	C-H Bend (Monosubstituted)	Alkenes (S)
986	C-H Bend (Monosubstituted)	Alkenes (S)
1045	C-O Stretch	Alcohols (S)(B)
1081	P-H Bend	Phosphines (M)
1109	C-C Stretch	Ketones (S)
1181	C-C(O)-C Stretch	Esters (M)
1255	C-O Stretch	Carboxylic Acid (S)
1306	C-O Stretch	Carboxylic Acid (S)
1384	N-O Stretch	Aliphatic Compounds (W)
1455	C-H Bend	Methyl (M-S)
1644	C=C Stretch (Isolated)	Alkenes (M)
1704	C=O Stretch	Carboxylic Acid (S)
2926	C-H Stretch	Methylene (M-S)
3403	N-H Stretch	Primary Amines (S)

M – Medium, S – Strong, W – Weak, B – Broad

Plants are important source of functional components for the development of new chemotherapeutic agents. Phytomedicine have been used for the treatment of diseases as in the cases of Unani and Ayurvedic system of medicines, a natural blueprint for the development of new drugs [29]. Analysis of the plant extracts from *H. suaveolens* and *S. campanulata* leaves revealed the presence of phytochemicals such as phenols, tannins, flavonoids, steroids, terpenoids, and simple sugars. This phytochemical investigation revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities [30]. All the extracts showed the presence of steroids and terpenoids for *H. suaveolens* and except in petroleum ether extract for *S. campanulata*. Steroids are responsible for cholesterol-reducing properties. Steroids also help in regulating the immune responses [31]. Steroids have also been reported to have antibacterial properties [32]. Terpenoids have been found to be useful in the prevention and therapy of several diseases, including cancer. Terpenoids are also known to possess antimicrobial, antifungal, antiparasitic, antiviral, anti-allergenic, antispasmodic, anti-hyperglycemic, anti-inflammatory and immunomodulatory properties [33,34]. The presence of tannins were indicated in both the plants except for petroleum ether extract. Flavonoids were also found in these plants signifying their role as a potent water-soluble antioxidant and free radical scavenger, which prevent oxidative cell damage and also have strong anticancer activity [35,36]. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. Tannins bind to proline rich protein and interfere with protein synthesis. Flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall [37]. The UV-VIS and FT-IR analysis is used for the identification of chemical constituents present in the aqueous, methanolic and acetone extracts of *H. suaveolens* and *S. campanulata* leaves. The results of the present study confirms the presence of amines, ethers, alkanes, alkenes, ketones, esters, carboxylic acids, alcohols, ketones, halogen, aliphatic and aromatic compounds in both the plants. Alkanes are known to protect the plant against water loss, prevent the leaching of important minerals by the rain, and protect against bacteria, fungi,

and harmful insects [38]. Alkynes are highly bioactive as nematocides [39]. Carboxylic acids are biologically very important in the formation of fat in the body. The drug aspirin is a carboxylic acid, and some people are sensitive to its acidity. The non-aspirin pain reliever ibuprofen is also a carboxylic acid [40]. The UV-VIS spectra for flavonoids typically lie in the range of 230-290 nm and the occurrence of peaks at 234-676 nm reveals the presents of phenolics (tannins) and alkaloids [41]. FT-IR is one of the most widely used methods to identify the chemical constituents and elucidate the compounds structures, and has been used as a requisite method to identify medicines in pharmacopoeia of many countries [42].

CONCLUSION

The preset study concludes the traditional use of *H. suaveolens* and *S. campanulata* for human ailments and their usage in herbal medicine as they are a rich source of phytochemicals with the presence of tannins, phenols, steroids, flavonoids and terpenoids. The presence of characteristic functional groups like ketones, alcohols, carboxylic acids, esters, alkanes and aromatic compounds supports the evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Hence, the above plant extracts could be explored for its highest therapeutic efficacy by pharmaceutical companies in order to develop safe drugs for various ailments. The traditional medical practices can be strongly endorsed for these plants as well as efforts should be geared up to further work out to isolate, purify and characterize the active constituents responsible for the activity of these plants. The quantitative analysis of these phytocompounds will be an interesting area for further study.

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