



Article

Chemistry

PHYTOCHEMICAL ANALYSIS OF *Terminalia chebula*

KESAVAN S

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College (Affiliated to Bharathidasan University) Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to

protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Terminalia chebula* (Tamil name: கடுக்காய்) seed.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Yellowish
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The seed powder of *Terminalia chebula* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (265.89 mg/gm) and flavonoids (90 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

S. No	Test name	Hydro-ethanolic extract
1	Tannin	++
2	Saponin	++
3	Flavonoids	++
4	Steroids	+
5	Terpenoids	++
6	Alkaloids	++
7	Antroquinone	+
8	Polyphenol	++
9	Glycoside	++
10	Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

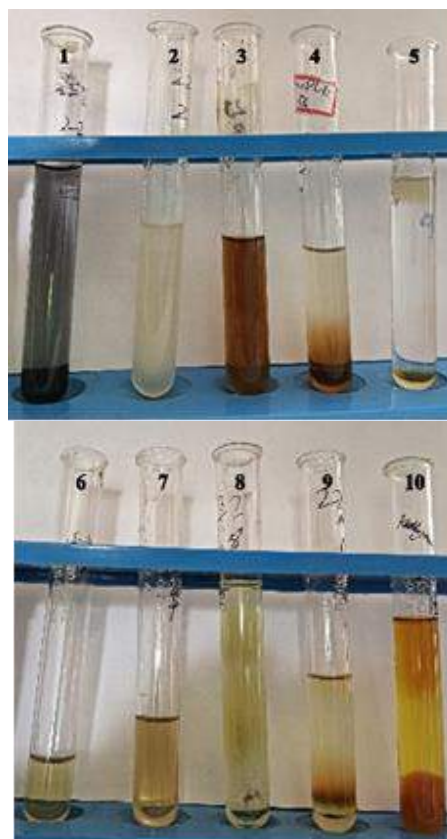


Plate 1: Qualitative screening of phytochemicals

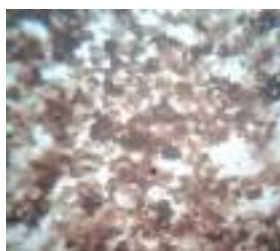
Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



Tannin

Flavonoids



Steroids

Polyphenol

Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Yellowish white	Pale green	Light yellow
2	Plant powder treated with distilled water	Brownish yellow	Yellowish green	Brown
3	Plant powder treated with Hexane	Yellowish brown	Light green	Brown
4	Plant powder treated with Chloroform	Pale yellow	Light green	Brown
5	Plant powder treated with Methanol	Brown	Dark green	Brown
6	Plant powder treated with Acetone	Brown	Pale green	Brown
7	Plant powder treated with 1N Sodium Hydroxide	Yellowish black	Greenish black	Greenish brown
8	Plant powder treated with 1N HCL	Yellowish green	Greenish brown	Black
9	Plant powder treated with sulphuric acid with equal volume of water	Black	Black	Black
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Golden yellow	Bright green	Black

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute

solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are

made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
390	Tannin
630	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	++
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica

gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationary phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.94).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Terminalia chebula* seed contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.

- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, *London New York*.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.



Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL SCREENING OF *Withania Somnifera*

SANTHOSHKUMAR R

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Withania Somnifera* (Tamil name: அஸ்வகந்தா) root.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Light tan
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The root powder of *Withania Somnifera* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (163.28 mg/gm) and flavonoids (70 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	++
Flavonoids	+
Steroids	+
Terpenoids	+
Alkaloids	+
Antroquinone	+

Polyphenol	++
Glycoside	++
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

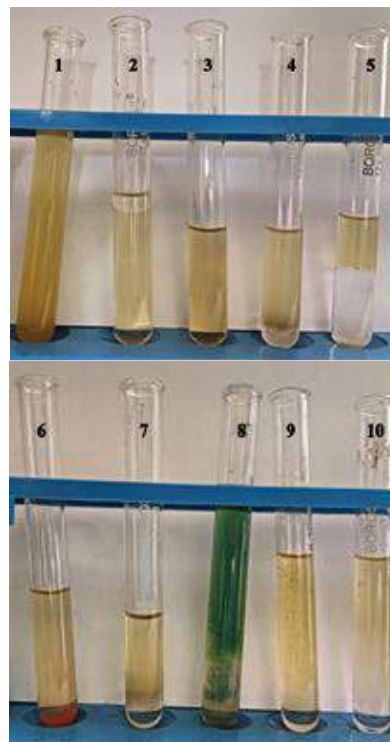


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	++
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



Tannin



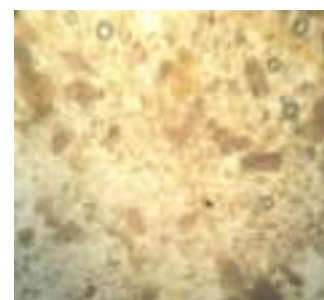
Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Yellow	Yellow	Brown
2	Plant powder treated with distilled water	Yellowish brown	Green	Brown
3	Plant powder treated with Hexane	Yellowish brown	Yellowish brown	Yellow
4	Plant powder treated with Chloroform	Brown	Yellowish brown	Yellow
5	Plant powder treated with Methanol	Light brown	Yellow	Green
6	Plant powder treated with Acetone	Light yellow	Brown	Yellow
7	Plant powder treated with 1N Sodium Hydroxide	Yellowish brown	Green	Green
8	Plant powder treated with 1N HCL	Yellow	Green	Brown
9	Plant powder treated with sulphuric acid with equal volume of water	Dark brown	Black	Green
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Yellowish brown	Green	Green

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
410	Tannin
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	+
4	Aldehydes	++
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat

surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.30).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Withania Somnifera* root contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.

- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, *London New York*.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL PROFILE OF *Glycyrrhiza glabra*

S. SHALINI

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids,

tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Glycyrrhiza glabra* (Tamil name: அதிமதுரம்) rhizome.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Brown
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The rhizome powder of *Glycyrrhiza glabra* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (232.00 mg/gm) and flavonoids (50.00 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	+
Flavonoids	+
Steroids	+
Terpenoids	+
Alkaloids	-
Antroquinone	-

Polyphenol	+
Glycoside	+
Coumarins	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present



Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	+
2	Flavonoids	+
3	Steroids	++
4	Terpenoids	++
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



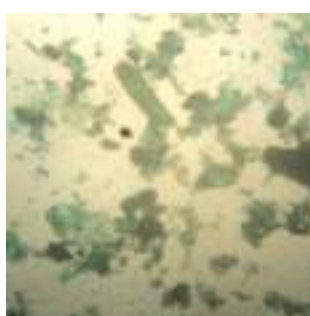
Tannin



Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Light yellow	Light yellow	Brown
2	Plant powder treated with distilled water	Light green	Greenish yellow	Brown
3	Plant powder treated with Hexane	Light green	Yellow	Dark black
4	Plant powder treated with Chloroform	Yellow brown	Green	Light black
5	Plant powder treated with Methanol	Yellow green	Brownish green	Black
6	Plant powder treated with Acetone	Brownish green	Light green	Brown
7	Plant powder treated with 1N Sodium Hydroxide	Dark brown	Dark green	Black
8	Plant powder treated with 1N HCL	Green	Dark green	Brown
9	Plant powder treated with sulphuric acid with equal volume of water	Brownish green	Dark brown	Dark brown
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Brown	Dark green	Dark black

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannins and Chlorophyll.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
390	Tannins
630	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	+
3	Aliphatic amines	+
4	Aldehydes	+
5	Ketones	+
6	Carboxylic acids	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate.

Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.74).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Glycyrrhiza glabra* rhizome contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant*

- Pigments. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, *London New York*.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause"s Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL EVALUATION OF *Aegle marmelos*

SIVARAMAKRISHNAN N

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Aegle marmelos* (Tamil name: வில்வம்) leaves.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Green
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The leaves powder of *Aegle marmelos* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (261.34 mg/gm) and flavonoids (10 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	++
Flavonoids	++
Steroids	++
Terpenoids	++
Alkaloids	++
Antroquinone	++
Polyphenol	++

Glycoside	++
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

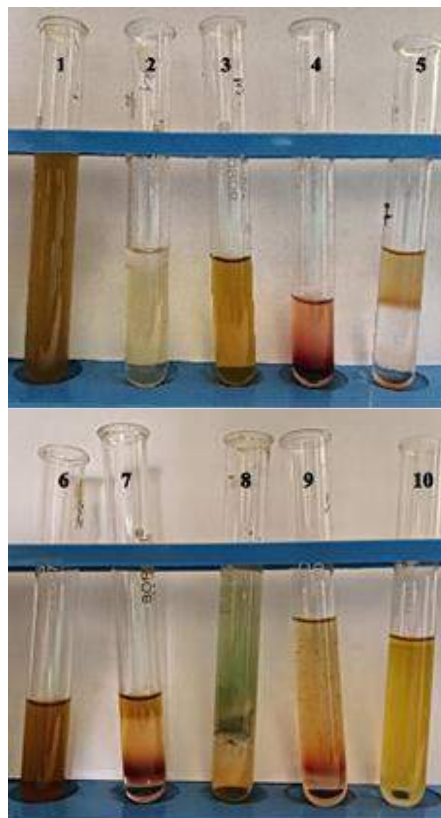


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	++
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present

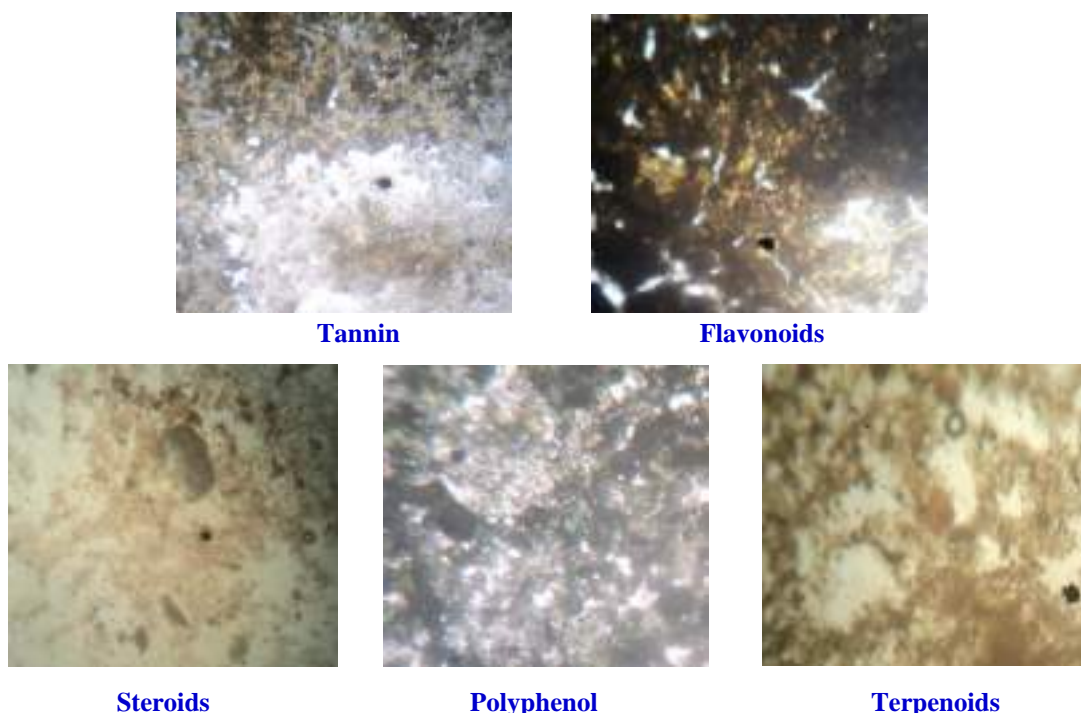


Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Yellow	Light green	Brown
2	Plant powder treated with distilled water	Black	Brown	Black
3	Plant powder treated with Hexane	Grey	Light green	Black
4	Plant powder treated with Chloroform	White	Light green	Black
5	Plant powder treated with Methanol	Black	Brown	Black
6	Plant powder treated with Acetone	Green	Light green	Black
7	Plant powder treated with 1N Sodium Hydroxide	Green	Yellowish green	Brown
8	Plant powder treated with 1N HCL	Brown	Light green	Black
9	Plant powder treated with sulphuric acid with equal volume of water	Black	Green	Black
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Dark brown	Yellowish green	Brown

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For

colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption

spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
360	Flavonoids
400	Tannin
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	+
4	Aldehydes	++
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were

detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.39).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Aegle marmelos* leaves contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S-757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.

- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant Caloncoba Echinata. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115-9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: Textbook of Pharmacognosy. (12th ed.). Balliese, Tindall and Co Publishers, London, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review Pharmacologyonline 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>**World Journal of Science and Research****Article****Chemistry****PHYTOCHEMICAL ASSESSMENT OF *Solanum trilobatum*****S. SNEKA**

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024**Revised on Aug. 2024****Online Nov. 2024****INTRODUCTION**

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Solanum trilobatum* (Tamil name: தூதுவளை) leaves.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Dark greenish
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The leaves powder of *Solanum trilobatum* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (216.07 mg/gm) and flavonoids (50.00 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	++
Flavonoids	++
Steroids	+
Terpenoids	+
Alkaloids	+

Antroquinone	-
Polyphenol	++
Glycoside	-
Coumarins	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

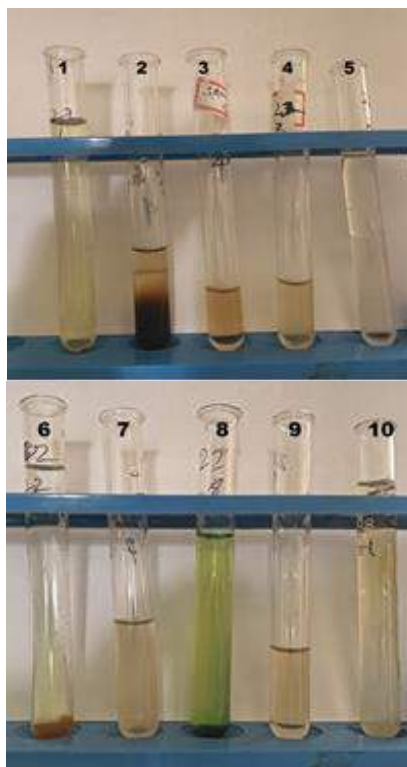


Plate 1: Qualitative screening of phytochemicals

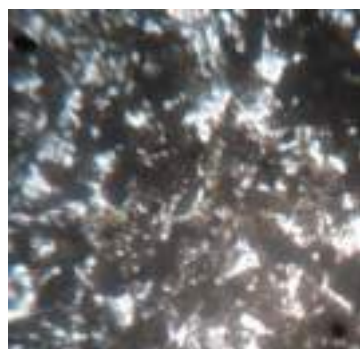
Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	+
2	Flavonoids	+
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

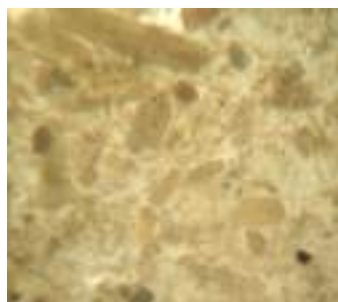
(+) Indicates Presence; (++) Moderately present



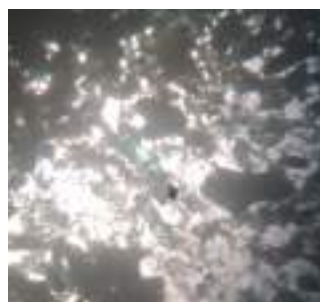
Tannin



Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Brownish	Brownish	Black
2	Plant powder treated with distilled water	Brownish	Greenish	Greenish
3	Plant powder treated with Hexane	Brownish	Brownish	Black
4	Plant powder treated with Chloroform	Greenish	Brownish	Greenish
5	Plant powder treated with Methanol	Black	Greenish	Black
6	Plant powder treated with Acetone	Yellowish	Brownish	Black
7	Plant powder treated with 1N Sodium Hydroxide	Yellowish	Greenish	Greenish
8	Plant powder treated with 1N HCL	Greenish	Greenish	Black
9	Plant powder treated with sulphuric acid with equal volume of water	Black	Black	Black
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Yellowish	Yellowish	Greenish

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an

automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is

400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of flavonols, Tannins and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
400	Tannins
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	+
4	Aldehydes	+
5	Ketones	+
6	Carboxylic acids	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots

by using spraying reagents Colored substances can be seen directly when viewed against the stationary phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.40).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Solanum trilobatum* leaves contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and*

- physiology. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL EVALUATION OF *Aloe vera*

SRINIVASAN P

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Aloe vera* (Tamil name: கற்பூரம்).

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Green
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The powder of *Aloe vera* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (221.18 mg/gm) and flavonoids (110 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	++
Saponin	++
Flavonoids	++
Steroids	++
Terpenoids	++
Alkaloids	++
Antroquinone	++
Polyphenol	++

Glycoside	++
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

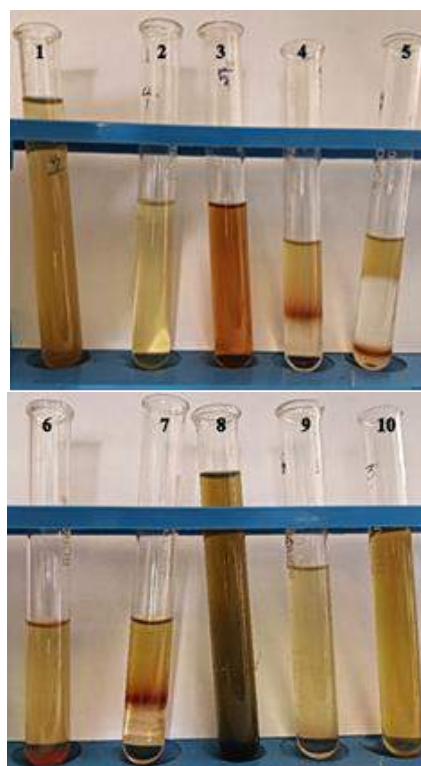


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

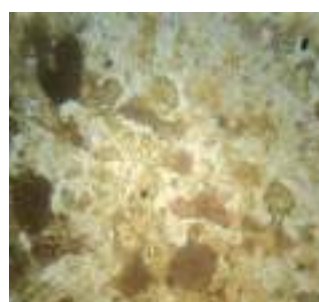
Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	++
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



Tannin



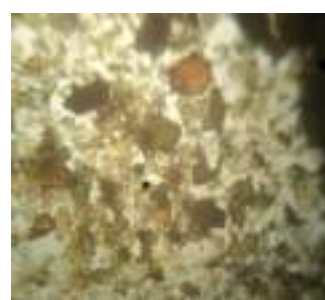
Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Brown	Yellowish brown	Light pink
2	Plant powder treated with distilled water	Brownish yellow	Yellow	Reddish brown
3	Plant powder treated with Hexane	Light brown	Light yellow	Black
4	Plant powder treated with Chloroform	Brownish yellow	Brownish yellow	Light red
5	Plant powder treated with Methanol	Dark brown	Greenish brown	Red
6	Plant powder treated with Acetone	Light yellowish brown	Greenish brown	Red
7	Plant powder treated with 1N Sodium Hydroxide	Reddish yellow	Greenish red	Reddish black
8	Plant powder treated with 1N HCL	Yellowish brown	Greenish brown	Yellowish red
9	Plant powder treated with sulphuric acid with equal volume of water	Dark brown	Greenish black	Yellowish red
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Yellowish black	Greenish black	Pinkish black

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an

automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is

400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
400	Tannin
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	++
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	-
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots

by using spraying reagents Colored substances can be seen directly when viewed against the stationary phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.88).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Aloe vera* contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and*

- physiology. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, Tindall and Co Publishers, London, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>**World Journal of Science and Research****Article****Chemistry****PHYTOCHEMICAL SCREENING OF *Sesbania grandiflora*****S. SWETHA**

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024**Revised on Aug. 2024****Online Nov. 2024****INTRODUCTION**

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Sesbania grandiflora* (Tamil name: அகத்தி கீரை) leaves.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Dark green
Odor	Characteristic smell
Taste	Bitterness
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The leaves powder of *Sesbania grandiflora* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (200.00 mg/gm) and flavonoids (40.00 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	+
Flavonoids	+
Steroids	+
Terpenoids	+
Alkaloids	+
Antroquinone	+

Polyphenol	++
Glycoside	-
Coumarins	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

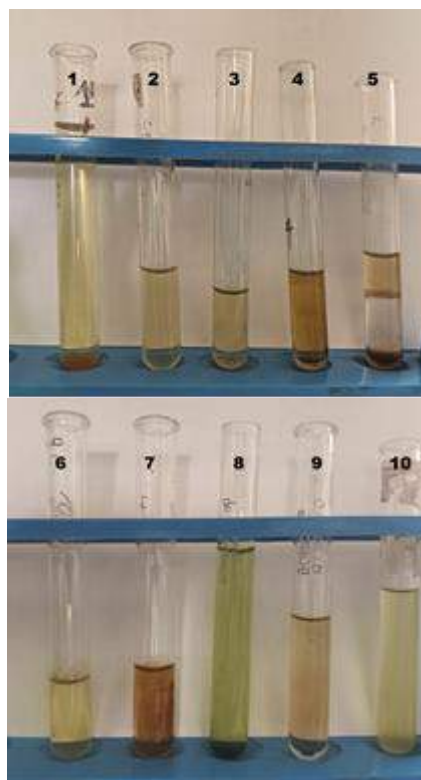


Plate 1: Qualitative screening of phytochemicals

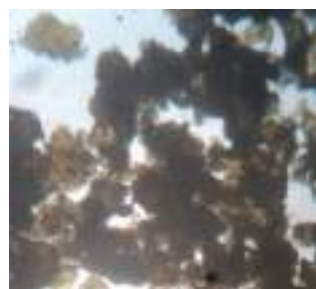
Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	+
2	Flavonoids	+
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



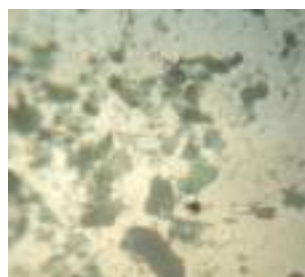
Tannin



Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Greenish	Greenish	Black
2	Plant powder treated with distilled water	Brownish	Greenish	Brownish
3	Plant powder treated with Hexane	Greenish	Greenish	Black
4	Plant powder treated with Chloroform	Greenish	Greenish	Black
5	Plant powder treated with Methanol	Brownish	Black	Black
6	Plant powder treated with Acetone	Greenish	Greenish	Greenish
7	Plant powder treated with 1N Sodium Hydroxide	Yellowish	Greenish	Greenish
8	Plant powder treated with 1N HCL	Greenish	Greenish	Black
9	Plant powder treated with sulphuric acid with equal volume of water	Black	Black	Black
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Yellowish	Brownish	Black

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are

made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical

density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannins and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
400	Tannins
640	Chlorophylls

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	+
5	Ketones	+
6	Carboxylic acids	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were

detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.89).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Sesbania grandiflora* leaves contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S-757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered

- vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115-9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, Tindall and Co Publishers, London, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>**World Journal of Science and Research****Article****Chemistry****PHYTOCHEMICAL ANALYSIS OF *Cynodon dactylon*****TAMILARASAND**

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024**Revised on Aug. 2024****Online Nov. 2024****INTRODUCTION**

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Cynodon dactylon* (Tamil name: அருகம்புல்) leaves.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Green
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The leaves powder of *Cynodon dactylon* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (128.16 mg/gm) and flavonoids (80 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	++
Saponin	++
Flavonoids	++
Steroids	+
Terpenoids	++
Alkaloids	++
Antroquinone	+

Polyphenol	++
Glycoside	+
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

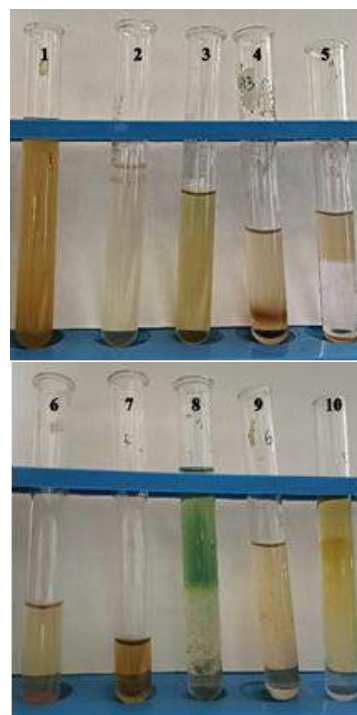


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

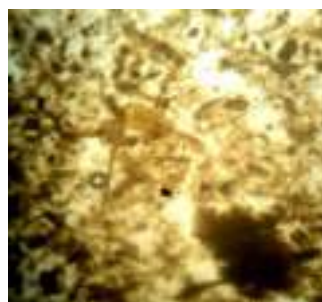
Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



Tannin



Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Green	Light green	Light green
2	Plant powder treated with distilled water	Green	Light yellow	Light black
3	Plant powder treated with Hexane	Light green	Brown	Light brown
4	Plant powder treated with Chloroform	Light yellow	Light green	Dark green
5	Plant powder treated with Methanol	Dark green	Light green	Green
6	Plant powder treated with Acetone	Light green	Light green	Light green
7	Plant powder treated with 1N Sodium Hydroxide	Light green	Light green	Green
8	Plant powder treated with 1N HCL	Green	Dark yellow	Dark green
9	Plant powder treated with sulphuric acid with equal volume of water	Green	Light green	Green
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Light green	Green	Dark green

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For

colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption

spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
400	Tannin
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	++
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	++
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots

by using spraying reagents Colored substances can be seen directly when viewed against the stationary phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.87).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Cynodon dactylon* leaves contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.

- Hasler CM and Blumberg JB (1999) Phytochemicals: Biochemistry and physiology. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant Caloncoba Echinata. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause"s Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem.* 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy.* (12th ed.). Balliese, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL CHARACTERIZATION OF *Glycyrrhiza glabra*

VEERAMANI D

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Glycyrrhiza glabra* (Tamil name: அதிமதுரம்) root.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Yellow
Odor	Characteristic smell
Taste	Slightly sweet
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The root powder of *Glycyrrhiza glabra* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (200.79 mg/gm) and flavonoids (50 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	++
Flavonoids	++
Steroids	++
Terpenoids	++
Alkaloids	+
Antroquinone	++
Polyphenol	++

Glycoside	++
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

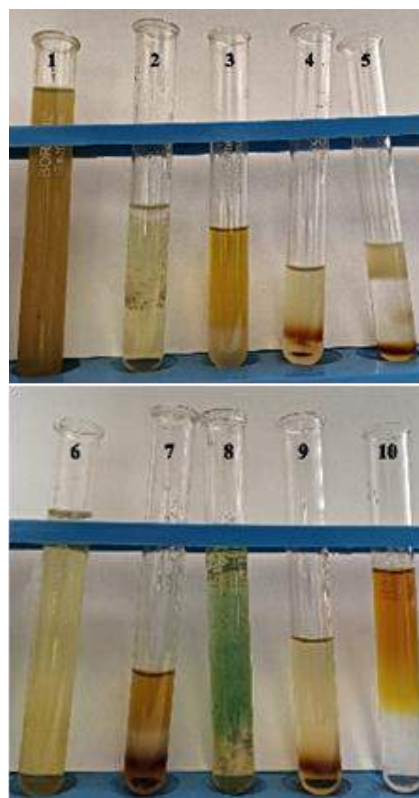


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present

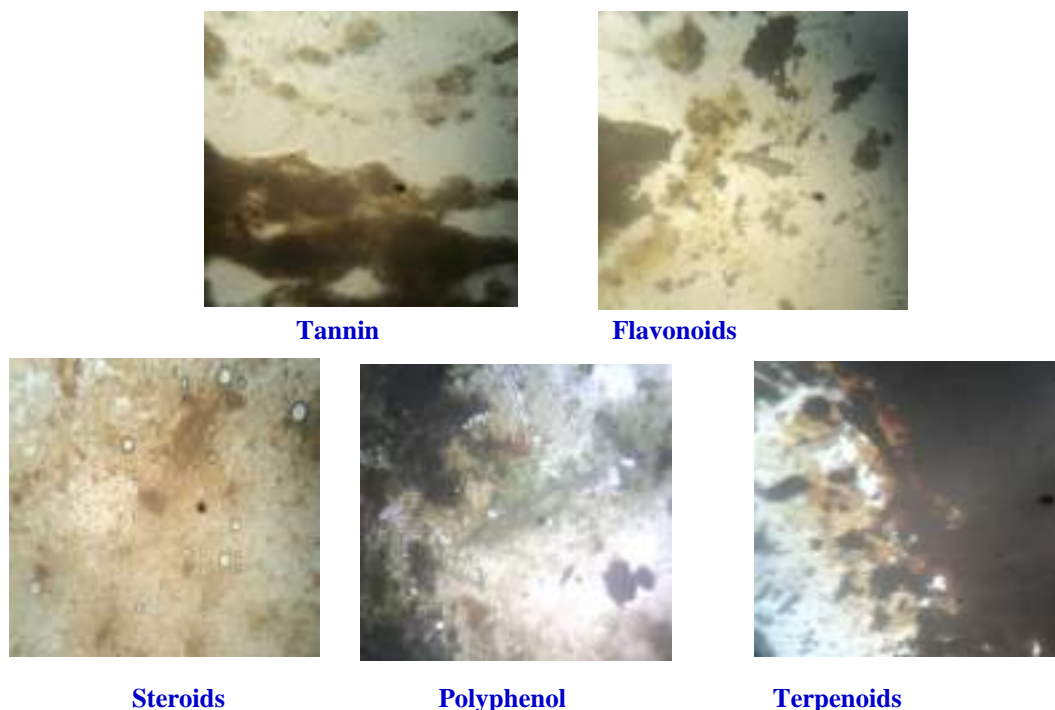


Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Light brown	Light green	Brown
2	Plant powder treated with distilled water	Light green	Brownish green	Black
3	Plant powder treated with Hexane	Brownish green	Light green	Light black
4	Plant powder treated with Chloroform	Light green	Green	Dark black
5	Plant powder treated with Methanol	Brown	Brown	Black
6	Plant powder treated with Acetone	Brownish green	Light brown	Black
7	Plant powder treated with 1N Sodium Hydroxide	Dark brown	Yellowish brown	Dark black
8	Plant powder treated with 1N HCL	Light green	Light green	Brown
9	Plant powder treated with sulphuric acid with equal volume of water	Dark green	Dark green	Light brown
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Brownish orange	Brown	Dark black

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For

colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption

spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
390	Tannin
630	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	+
4	Aldehydes	+
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the

stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.74).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Glycyrrhiza glabra* root contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S-757S.

- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliere, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>**World Journal of Science and Research****Article****Chemistry****PHYTOCHEMICAL PROFILE OF *Trachyspermum ammi*****A. VERRONIKKAM**

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024**Revised on Aug. 2024****Online Nov. 2024****INTRODUCTION**

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Trachyspermum ammi* (Tamil name: ஓமம்/ அஜ்வைன்) leaves.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Dark green
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The leaves powder of *Trachyspermum ammi* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (362.00 mg/gm) and flavonoids (70.00 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	+
Saponin	++
Flavonoids	++
Steroids	+
Terpenoids	++
Alkaloids	+

Antroquinone	+
Polyphenol	++
Glycoside	-
Coumarins	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

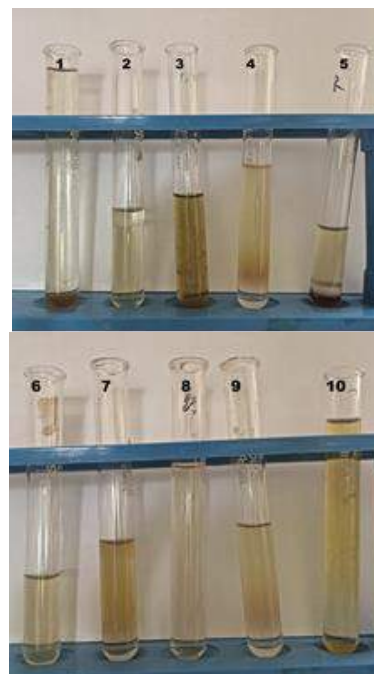


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	+
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present



Tannin



Flavonoids



Steroids



Polyphenol



Terpenoids

Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Green	Brown	Green
2	Plant powder treated with distilled water	Green	Brown	Green
3	Plant powder treated with Hexane	Light green	Brown	Light brown
4	Plant powder treated with Chloroform	Light green	Brown	Green
5	Plant powder treated with Methanol	Yellow	Brown	Dark green
6	Plant powder treated with Acetone	Dark brown	Black	Dark green
7	Plant powder treated with 1N Sodium Hydroxide	Light yellow	Brown	Light brown
8	Plant powder treated with 1N HCL	Brown	Black	Dark green
9	Plant powder treated with sulphuric acid with equal volume of water	Light brown	Black	Light green
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Yellowish orange	Black	Green

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 340 to 400 nanometres

(nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important

in the identification of many plant constituents, crude plant extracts for the presence of flavonols, Terpenoids and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
420	Terpenoids
640	Chlorophylls

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	+
2	Phenol	++
3	Aliphatic amines	+
4	Aldehydes	+
5	Ketones	+
6	Carboxylic acids	+

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationary phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the

regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.89).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Trachyspermum ammi* leaves contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S-757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet

- radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115-9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliere, Tindall and Co Publishers, London, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL PROFILE OF *Leucas aspera*

VIJAYAKUMAR S

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary

metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Leucas aspera* (Tamil name: துளம்பை) leaves.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Green
Odor	Characteristic smell
Taste	Bitter
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The leaves powder of *Leucas aspera* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (215.63 mg/gm) and flavonoids (50 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	++
Saponin	++
Flavonoids	++
Steroids	+
Terpenoids	++
Alkaloids	++
Antroquinone	++
Polyphenol	++

Glycoside	++
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

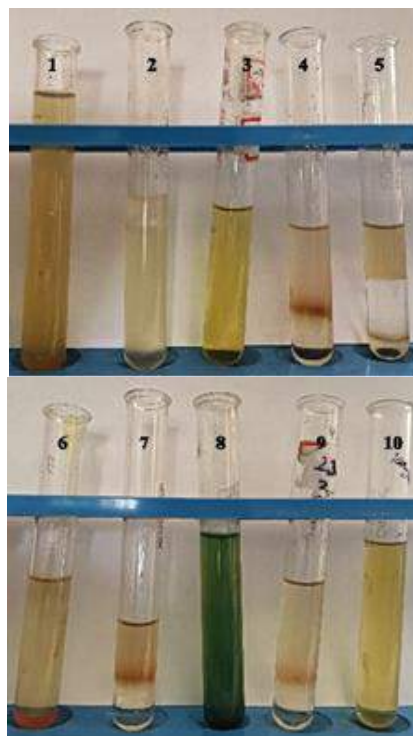


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present

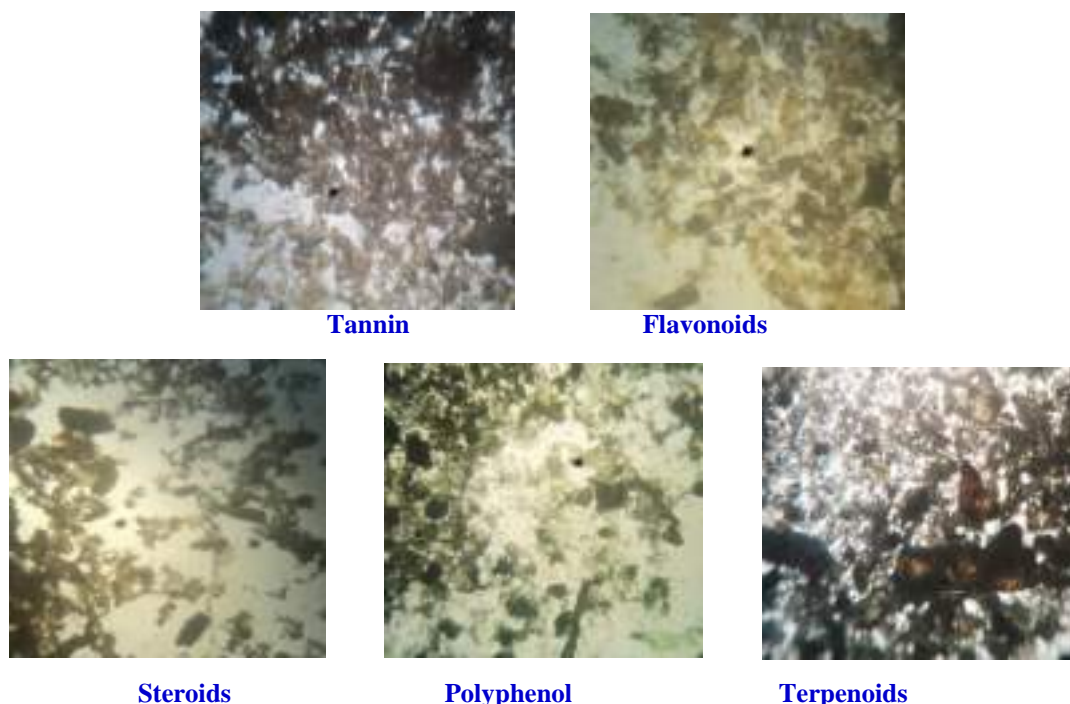


Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Brownish white	Light green	Black
2	Plant powder treated with distilled water	Light brown	Green	Black
3	Plant powder treated with Hexane	Brownish white	Light green	Black
4	Plant powder treated with Chloroform	Light brown	Green	Black
5	Plant powder treated with Methanol	Brown	Green	Brownish black
6	Plant powder treated with Acetone	Brown	Green	Black
7	Plant powder treated with 1N Sodium Hydroxide	Brownish green	Dark brown	Black
8	Plant powder treated with 1N HCL	Greenish yellow	Light green	Brownish green
9	Plant powder treated with sulphuric acid with equal volume of water	Brownish green	Light green	Black
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Brownish green	dark green	Brownish green

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For

colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption

spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
400	Tannin
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	++
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	+
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate. Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the

stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.86).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Leucas aspera* leaves contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant Pigments*. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, London New York.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S-757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet

- radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115-9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliere, Tindall and Co Publishers, London, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.

Available online at <http://www.harmanpublications.com>

World Journal of Science and Research



Article

Chemistry

PHYTOCHEMICAL SCREENING OF *Nelumbo nucifera*

VIJEYAN S

Department of Chemistry, H.H. The Rajah's College (Autonomous), An Autonomous College Affiliated to Bharathidasan University Accredited by NAAC with 'B' Grade, Madurai Road, Pudukkottai - 622 001 Tamil Nadu.

Received on July 2024

Revised on Aug. 2024

Online Nov. 2024

INTRODUCTION

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Mathai, 2000). Recently, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged and are classified by protective function, physical characteristics and chemical characteristics (Meagher and Thomson, 1999). Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

The secondary metabolites formed also are an important trait for our food plants (taste, colour, scent, etc.) and ornamental plants. Moreover, numerous plant secondary metabolites such as flavonoids, alkaloids,

tannins, saponins, steroids, anthocyanins, terpenoids, rotenoids etc. have found commercial application as drug, dye, flavour, fragrance, insecticide, etc. Such fine chemicals are extracted and purified from plant materials. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases including cancer, cardiovascular, arthritis, diabetic, aging etc (Velavan, 2011). The techniques application of chromatography, mass spectrometry, infrared spectrometry, and nuclear magnetic resonance allowed quantitative and qualitative measurements of a large number of plant metabolites (Velavan, 2015).

Phytochemical screenings are preliminary tests conducted to detect the presence of both primary and secondary metabolites in an extract. Several qualitative analyses described below have been used to detect the presence of secondary metabolite like alkaloids, flavonoids, tannins, saponins, flavones, sterols, terpenes, cardiac glycosides while primary metabolites like protein, carbohydrates, and fats (Pandey and Tripathi, 2014; Beena *et al.*, 2016; Trease and Evans, 1989). This internship mainly deals with the general techniques involved in phytochemical analysis from plant source. The chosen plant was *Nelumbo nucifera* (Tamil name: தாமரை) flower.

TECHNIQUES AND OBSERVATION

Organoleptic character refers to evaluation each of the powdered traditional medicinal plant by colour, odour, taste, texture and shape (Koroma *et al.*, 2022). These criteria therefore should be considered aspects of raw material 'quality'. The responses for organoleptic characters are given in Table 1. The comparative study for organoleptic parameters such as color, taste, odour, texture and shape which confirm the overall acceptability

Table 1: Organoleptic Characters of herbal

Characters	Results
Colour	Brownish pink
Odor	Characteristic smell
Taste	Light sweet
Texture	Fine powder
Shape	Uneven crystal

Phytochemicals extraction and identification

The flower powder of *Nelumbo nucifera* were purchased in June 2024 from Thanjavur district, Tamil Nadu, India. 1 gram of the powder transferred in to different conical flask (250ml). The conical flask containing 50ml of hydro-ethanol solvent. The conical flask containing sample were shaken well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using Whatman filter paper No.1 and filtrate is used for further analysis. Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Harborne (1973 and 1976). Table 2 shows the presence of phytochemicals in hydro-ethanolic extract (Plate 1) and significant amounts of total phenol (236.22 mg/gm) and flavonoids (50 mg/gm) were reported.

Table 2: Qualitative screening of phytochemicals

Test name	Hydro-ethanolic extract
Tannin	++
Saponin	++
Flavonoids	++
Steroids	+
Terpenoids	++
Alkaloids	++
Antroquinone	++
Polyphenol	++

Glycoside	++
Coumarins	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

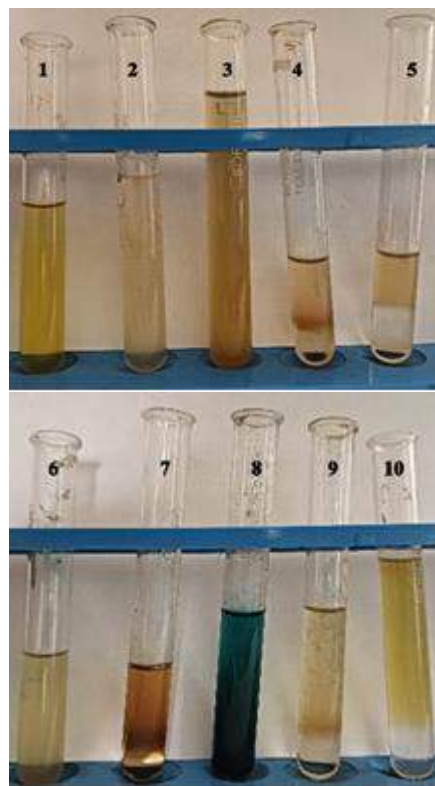


Plate 1: Qualitative screening of phytochemicals

Histochemical analysis

Histochemical analysis of plant powder were investigated. The different reagents treated with plant powder and observed under microscope. The histochemicals results revealed the presence of tannin, flavonoids, polyphenol, Steroids and terpenoids in plant. In this results further confirmed the presence of phytochemicals in plant (Table 3 and Plate 2).

Table 3: Histochemical analysis

S. No	Phytochemicals	Results
1	Tannin	++
2	Flavonoids	++
3	Steroids	+
4	Terpenoids	+
5	Polyphenol	++

(+) Indicates Presence; (++) Moderately present

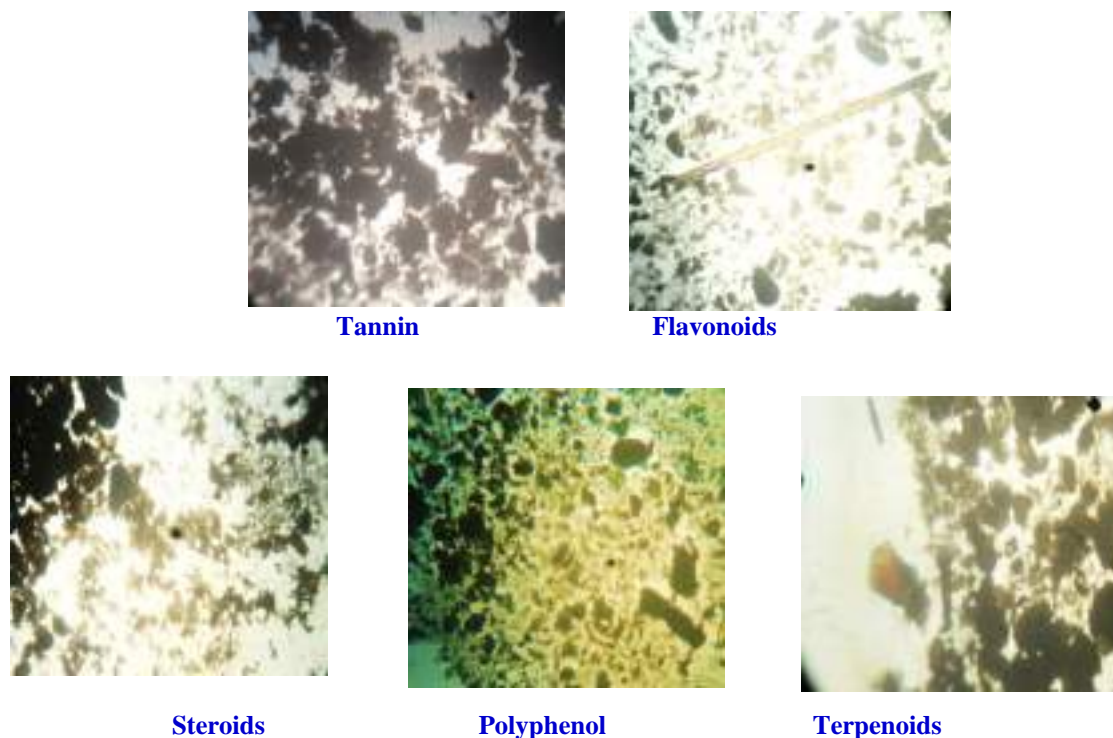


Plate 2: Histochemical analysis

Fluorescence analysis

Fluorescence analysis of entire of plant has been carried out in daylight and under UV light (Kokashi *et al.*, 1957). Fluorescence analysis of powder of plant was carried out by the treatment of different chemical reagents such as H₂O, H₂SO₄, HCl, NH₃, HNO₃, CH₃OH and NaOH. The

fluorescence color shows specificity for each compound. It acts as a preliminary pharmacognostic parameter for identification and standardization of a particular drug from its adulterants. The Fluorescence behavior presence of the major bioactive compounds in the crude plant was found to be phenols, flavonoids, steroids and Coumarins (Table 4).

Table 4: Fluorescence analysis

S. No	Test	Colour observation		
		Visible Light	Short UV Light (254 nm)	Long UV Light (365 nm)
1	Plant powder	Brownish white	Brown	Black
2	Plant powder treated with distilled water	Brownish green	Brownish green	Black
3	Plant powder treated with Hexane	Brown	Brown	Black
4	Plant powder treated with Chloroform	Brownish white	Light brown	Black
5	Plant powder treated with Methanol	Light brown	Brown	Black
6	Plant powder treated with Acetone	Dark brown	Light brown	Black
7	Plant powder treated with 1N Sodium Hydroxide	Brownish white	Dark brown	Black
8	Plant powder treated with 1N HCL	Brown	Brownish green	Dark green
9	Plant powder treated with sulphuric acid with equal volume of water	Greenish yellow	Dark brown	Black
10	Plant powder treated with HNO ₃ diluted with an equal volume of water	Dark brown	Brown	Black

Ultraviolet-visible spectroscopy analysis

The absorption spectra of plant constituents can be measured in very dilute solution against a solvent blank using an automatic recording spectrophotometer. For colourless compounds, measurements are made in the range 340 to 400 nanometres (nm); for coloured compounds, the range is 400 to 700 nm. The wavelengths of the maxima and minima of the absorption spectrum so obtained are recorded (in nm) and also the intensity of the absorbance (or optical density) at the particular maxima and minima (Table 5) spectral measurements are important in the identification of many plant constituents, crude plant extracts for the presence of Flavonoids, Tannin and Chlorophylls.

Table 5: Ultraviolet-visible spectroscopy analysis

Wavelength (nm)	*Compound identified (Harborne, 1973)
340	Flavonoids
400	Tannin
640	Chlorophyll

Detection of functional groups

Functional groups were detected by the method of Ahluwalia and Dhingra, (2004). A functional group is a group of atoms or bonds inside a substance that is responsible for the substance's unique chemical reactions. Qualitative functional groups analysis is used to determine the functional groups of in a molecule. This study was carried out to screen qualitative analysis of functional group present in the plant extract of plant. Table 6 represent the qualitative a functional groups analysis in plant.

Table 6: Analysis of functional groups

S. No	Functional groups	Results
1	Alcohols	++
2	Phenol	++
3	Aliphatic amines	++
4	Aldehydes	+
5	Ketones	++
6	Carboxylic acids	++

(-) Indicates Absence; (+) Indicates Presence; (++) Moderately present

Thin Layer Chromatography (Harborne, 1973).

Thin layer Chromatography is based upon the principles of column and partition Chromatography. A thin layer of the stationary phase is formed on a suitable flat surface, such as glass and plastic plate.

Separation of a mixture in this case is achieved over a thin layer of alumina or silica gel to which they are absorbed by different physical forces. TLC plate prepared according to the standard procedure. (Harborne, 1973; Stahl, 1969). The presences of phytochemicals in the extracts were detected by TLC using suitable spraying reagents. Detection of spots by using spraying reagents Colored substances can be seen directly when viewed against the stationery phase whilst colorless species were detected by spraying the plate with appropriate reagent, which produced coloured areas in the regions, which they occupy (Harborne, 1973). The TLC report showed in the Plate 3 (Rf = 0.77).



Plate 3: Thin Layer Chromatography

SUMMARY

Overall, I found that *Nelumbo nucifera* flower contain rich sources of phytochemicals identified by various phytochemical techniques. A rich source of various phytochemicals widely used in phyto-pharmacological research for health-promoting activity.

REFERENCES

- Ahluwalia, V. K., & Dhingra, S. (2004). *Comprehensive Practical Organic Chemistry: Qualitative Analysis*. Universities Press.
- Banu, K. S., & Cathrine, L. (2015). General techniques involved in phytochemical analysis. *International journal of advanced research in chemical science*, 2(4), 25-32.
- Beena P, Rajesh KJ, Arul B. Preliminary phytochemical screening of *Cicer arietinum* in folklore medicine for hepatoprotection. *J Innov Pharm Biol Sci*. 2016;3:153-9.
- Harborne JB. Functions of flavonoids in plants. In: Goodwin TW, editor. *Chemistry and Biochemistry of Plant*

- Pigments. New York: Academic Press; 1976. p. 736-78.
- Harborne, J. B. (1973). *Phytochemical Methods; A guide to modern techniques of plant Analysis*. 2nd Edition, *London New York*.
- Hasler CM and Blumberg JB (1999) *Phytochemicals: Biochemistry and physiology*. Introduction. *Journal of Nutrition* 129: 756S–757S.
- Kokashi CJ, Kokashi RJ and Sharma M. (1957) Fluorescence of powdered vegetable drugs in ultra- violet radiation. *J American Pharm Assoc* 47:715-717.
- Koroma, L., Kpaka, J., & Kpaka, A. (2022). Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant *Caloncoba Echinata*. *International Journal of Innovation in Engineering*, 2(3), 14-22.
- Mathai K(2000). Nutrition in the Adult Years. In Krause's Food, Nutrition, and Diet Therapy, 10th ed., ed. L.K. Mahan and S. Escott-Stump; 271: 274-275.
- Pandey A, Tripathi S. Concept of standardization, extraction, and pre-phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014;2:115–9.
- Stahl, E. (1969). Apparatus and general techniques in TLC. In *Thin-Layer Chromatography: A Laboratory Handbook* (pp. 52-86). Berlin, Heidelberg: Springer Berlin Heidelberg.
- Trease, G. E., & Evans, W. C. (1989). Phenols and Phenolic glycosides. In: *Textbook of Pharmacognosy*. (12th ed.). Balliese, *Tindall and Co Publishers, London*, 343-383.
- Velavan S. (2011) Free radicals in health and diseases —A mini review *Pharmacologyonline* 1: 1062-1077 (2011) Newsletter.
- Velavan, S. "Phytochemical techniques-a review." *World Journal of Science and Research* 1.2 (2015): 80-91.