IRSTI 31.27.21; 31.23.01 https://doi.org/10.26577/IJBCh2024v17.i2.13

A.E. Johns [1]*, P.M. Radhamany[2]

[1] St. Thomas College, Kozhencherry, Kerala, India
[2] University of Kerala, Thiruvananthapuram Kariavattom, Kerala, India
*e-mail: adheenajohns123@gmail.com
(Received 20 October 2024; received in revised form 16 November 2024; accepted 24 November 2024)

Phytochemical Screening, HPTLC and FT-IR analysis of methanolic bark extract of *Syzygium stocksii* (Duthie) Gamble – a critically endangered taxon in Myrtaceae

Abstract. *Syzygium* is a large genus with numerous species in the Family Myrtaceae. The genus *Syzygium* is a treasure trove of phytochemicals with immene therapeutic potential. *Syzygium stocksii* (Duthie) Gamble syn. *Syzygium travancoricum*, a critically endangered plant collected from Thrissur District in Kerala, India. The present study aims at the preliminary phytochemical screening, HPTLC and FT-IR analysis of the plant. Qualitative phytochemical studies revealed the presence of alkaloids, phenolics, flavonoids, tannins, and terpenoids. The preliminary phytochemical screening results revealed the presence of more constituents on the methanolic bark extract, and hence, this was further subjected to an HPTLC analysis to determine the number of compounds in the crude extract. FTIR analysis was also conducted to identify the major functional groups in the compounds in the extract. From this study, it can be concluded that *Syzygium stocksii* contains various bioactive compounds. This study calls for the importance of excavating the phytochemical and pharmacological potential of this relatively unexplored species.
Key words: *Syzygium stocksii*, preliminary phytochemical screening, HPTLC, FTIR.

Introduction

The genus *Syzygium*, part of the Myrtaceae family, is the largest woody genus of flowering plants, comprising 1,200 to 1,800 species found across the Old-World tropics and subtropics [1]. Myrtaceae plants are well-known for their rich supply of medicinally valuable volatile oils, which are widely used in traditional medicines throughout various ethnobotanical practices in tropical regions [2]. Numerous phytochemical studies have been conducted on various *Syzygium* species, such as *Syzygium cumini*, *S. jambos*, *S. malaccense*, *S. guineense*, and *S. caryophyllatum* [3,4].

Among the species, *Syzygium stocksii* (Duthie) Gamble (syn. *Syzygium travancoricum* Gamble) remains relatively unexplored despite its significant ethnopharmaceutical importance. This fruit tree, classified as critically endangered under the IUCN Threatened Species category, is reported to have only around 200 trees remaining in the Western Ghats according to the IUCN Red List (2010, 2012, 2013). *Syzygium stocksii* is an evergreen tree that can grow up to 25 meters in height with white flowers, typical of the Myrtaceae family. While many studies have evaluated the phytopharmacological properties of other *Syzygium* species, research on *Syzygium stocksii* is still sparse.

Materials and methods

Plant material and sample preparations. Leaves, bark, fruit pulp, and seeds of *S. stocksii* were collected from Thrissur district, Kerala, India. A voucher specimen (KUBH 10252) was prepared, identified and deposited at the Department of Botany, University of Kerala. For phytochemical analysis, bark, leaves, fruit pulp and seeds were separately dried at below 40°C. The powdered samples were sequentially extracted with hexane, ethyl acetate, acetone, methanol, and water in the increasing polarity with the Soxhlet apparatus. The extracts were filtered through Whatman No.1 filter paper and the solvents were evaporated under reduced pressure. Dried extracts obtained were stored at 4°C until further analysis.

Preliminary phytochemical screening. Qualitative phytochemical analyses were done for establishing a profile of the given extract for its chemical composition. The following tests were performed on extracts to detect various phytoconstituents present in them [6-8]:

Detection of alkaloids. Stirred 50 mg solvent free extract with few ml of dilute hydrochloric acid and filtered. The filtrate was tested carefully with various alkaloid reagents as follows.

Mayer's test. To a few ml of filtrate, one or two drops of Mayer's reagent were added through the side of the test tube. A white or creamy precipitate indicates the test as positive.

Dragendorff's test. To a few ml of filtrate, 1 or 2 ml of Dragendorff's reagent was added. A prominent yellow precipitate indicates the test as positive.

Detection of anthocyanins. The addition of 10% NaOH to the extract resulted in a blue coloration, indicating the presence of anthocyanins. Similarly, the addition of concentrated sulfuric acid produced a yellowish orange colour, further confirming the presence of anthocyanins.

Detection of coumarins. To the extract, addition of alcoholic KOH or NaOH resulted yellow colouration appears which will disappear on adding concentrated HCl indicating the presence of coumarins.

Detection of flavonoids. Three different methods were employed to determine the presence of flavonoids in the plant samples. For the alkaline reagent test, the extract was treated with a few drops of NaOH solution, resulting in the formation of an intense yellow colour. This yellow colour disappeared upon the addition of a dilute acid, confirming the presence of flavonoids. To a portion of the aqueous filtrate of the extract, 5 ml of dilute ammonia solution was added, followed by concentrated sulfuric acid. The appearance of a yellow coloration in the extract indicates the presence of flavonoids. A portion of the filtrate was heated with 10 ml of ethyl acetate over a steam bath for 3 minutes. The mixture was filtered and 4 ml of filtrate was shaken with 1 ml of dilute ammonia solution. Yellow coloration indicates the presence of flavonoids.

Detection of glycosides. Fifty milligrams of the extract were hydrolysed with concentrated hydrochloric acid for 2 hours on a water bath. After filtration, the hydrolysate was subjected to Bontrager's test [9]. To 2 ml of the filtered hydrolysate, 3 ml of chloroform was added and shaken. The chloroform layer was then separated, and 10% ammonia was added to it. The appearance of a pink colour indicated the presence of glycosides.

Detection of phenolic compounds. Ferric chloride test. Dissolved 50mg of extract in 5ml of distilled water and to which few drops of neutral 5% ferric chloride solution was added. Dark green colour indicates the presence of phenolic compounds.

Gelatin test. The extract (50mg) was dissolved in 5ml of distilled water and added 2ml of 1% solution of gelatin containing 10% sodium chloride to it. White precipitate indicates the presence of phenolic compounds.

Detection of saponins. Froth test. The extracts were diluted with distilled water to a total volume of 20 ml and shaken in a graduated cylinder for 15 minutes. The formation of a foam layer over 1cm indicates the presence of saponins [10].

Foam test. A total of 0.5 g of the extract was shaken with 2 ml of water. The persistence of foam for ten minutes indicated the presence of saponins.

Detection of Steroids. Libermann-Burchrd's test. Added 2 ml of acetic anhydride to 0.5 g extract of each sample with 2 ml of sulphuric acid. The colour change from violet to blue or green indicates the presence of steroids.

Detection of tannins. Gelatin test. To the extract, 1% gelatin solution containing sodium chloride was added. The formation of a white precipitate indicated the presence of tannins. Additionally, the extract was treated with ferric chloride, and the appearance of a dark blue or greenish-black color further confirmed the presence of tannins.

Detection of terpenoids. Salkowski test. Mixed 5 ml of the extract with 2 ml of chloroform and carefully added 3 ml of concentrated sulphuric acid to form a layer. A reddish-brown coloration at the interface was formed to show a positive result for the presence of terpenoids.

Detection of quinone. The extract is mixed with concentrated sulphuric acid; the resulting red colour indicates the presence of quinone.

Detection of carbohydrates and reducing sugar. Dissolved small quantities of the filtrate in 4ml of distilled water and filtered. The filtrate was subjected to Fehling's test. The extract was treated with Fehling's reagent A & B. The appearance of reddish-brown colour precipitate indicates the presence of reducing sugar. For the Benedict's test, the extract was treated with Benedict's reagent. Appearance of reddish orange colour precipitate indicates the presence of reducing sugar.

Detection of iridoids. The extract was added to 1 ml of reagent (10 ml acetic acid, 0.2% $CuSO_4$, and 0.5 ml conc. HCl). The mixture was heated over a small flame. The development of a light blue colour indicates the presence.

High Performance Thin Layer Chromatography Analysis (HPTLC). A number of solvent systems were tried and a system which gave the maximum resolution was ethyl acetate: methanol (5:0.8) and

was selected as the solvent system for the extract. The extracts were applied as different tracks of different concentrations of width 8 mm each on silica gel 60 F 254 pre-coated aluminium sheets through CAMAG micro litre syringe using Automatic TLC Sampler 4 (ATS4). After sample application the plate was introduced vertically in a CAMAG developing chamber (10 cm × 10 cm) pre-saturated with the mobile phase selected. The developed chromatogram was air dried to evaporate solvents from the plate and the plate was kept in CAMAG Vizualizer and the images were captured under UV light at 254 nm and 366 nm. The plate was scanned at 254 nm and 366 nm using TLC Scanner 4 and the fingerprint profiles were documented. The R f values and finger print data were recorded with winCATS software associated with the scanner. The plate was derivatised using vanillin-sulphuric acid reagent, heated at 105^0C by placing on CAMAG TLC plate heater till the colour of the bands appeared. Then the plate was visualized under white light and the chromatograms were documented. The plate was scanned at 575 nm and the R_f values and finger print data were documented.

Fourier Transformed Infrared Spectroscopy (FTIR) Analysis. About 1mg of the dried methanolic extract was encapsulated in 10 mg of KBr pellet in order to prepare translucent sample discs. The powdered sample of the extract was loaded in FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The spectral data was taken on an Agilent Cary 630 FTIR spectrometer based on the ATR (Attenuated Total Reflection) method. This was done to determine the functional groups in the plant extract.

Results and discussion

The results of the preliminary phytochemical analysis, as presented in Tables 1-4, indicate the presence of alkaloids, phenols, terpenoids, and flavonoids in the leaves, bark, fruit pulp, and seeds. Among the solvents tested – hexane, ethyl acetate, acetone, distilled water, and methanol – methanol extracts revealed the highest number of phytochemicals, while hexane extracts showed the least. Different plant parts, including leaves, bark, fruit, and seeds, were found to contain multiple phytochemical compounds, many of which are recognized for their beneficial roles in medical sciences.

Table 1 – Preliminary phytochemical screening of the leaves of *Syzygium stocksii*

Phytochemical constituents	Hexane	Ethyl acetate	Acetone	Methanol	Distilled water
Alkaloids	-	-	-	+	-
Anthocyanins	-	-	-	+	-
Coumarins	+	+	+	+	-
Flavonoids	-	-	+	+	+
Glycosides	-	-	+	+	+
Phenols	-	+	+	+	+
Saponins	-	-	-	+	+
Steroids	-	-	-	-	-
Tannins	-	+	+	+	+
Terpenoids	-	-	-	+	-
Quinones	-	-	-	-	-
Reducing sugar	-	-	-	-	+
Carbohydrates	-	-	-	-	+
Iridoids	+	-	-	-	-
Note: "+"indicates the presence of phytochemical; "-" indicates the absence of phytochemical					

Table 2 – Preliminary phytochemical screening of the bark of *Syzygium stocksii*

Phytochemical constituents	Hexane	Ethyl acetate	Acetone	Methanol	Distilled water
Alkaloids	-	-	-	+	-
Anthocyanins	-	-	-	+	-
Coumarins	+	+	+	+	-
Flavonoids	-	-	+	+	+
Glycosides	-	-	+	+	+
Phenols	-	+	+	+	+
Saponins	-	-	+	+	+
Steroids	-	-	-	+	-
Tannins	-	+	+	+	+
Terpenoids	-	-	-	+	-
Quinones	-	-	-	-	-
Reducing sugar	-	-	+	-	+
Carbohydrates	-	-	+	-	+
Iridoids	-	-	-	-	-
Note: "+"indicates the presence of phytochemical; "-" indicates the absence of phytochemical					

Table 3 – Preliminary phytochemical screening of the fruit pulp of *Syzygium stocksii*

Phytochemical constituents	Hexane	Ethyl acetate	Acetone	Methanol	Distilled water
Alkaloids	-	-	-	+	+
Anthocyanins	-	-	-	-	+
Coumarins	+	+	+	+	-
Flavonoids	-	-	+	+	+
Glycosides	-	-	+	+	-
Phenols	-	+	+	+	+
Saponins	-	-	-	+	-
Steroids	-	-	-	-	-
Tannins	-	+	+	+	+
Terpenoids	+	+	+	+	+
Quinones	-	-	-	-	-
Reducing sugar	-	-	-	+	-
Carbohydrates	-	-	+	+	+
Iridoids	-	-	-	-	-
Note: "+"indicates the presence of phytochemical; "-" indicates the absence of phytochemical					

Table 4 – Preliminary phytochemical screening of the seeds of *Syzygium stocksii*

Phytochemical constituents	Hexane	Ethyl acetate	Acetone	Methanol	Distilled water
Alkaloids	-	-	-	+	+
Anthocyanins	+	-	-	-	-
Coumarins	+	+	-	+	-
Flavonoids	-	-	+	+	+

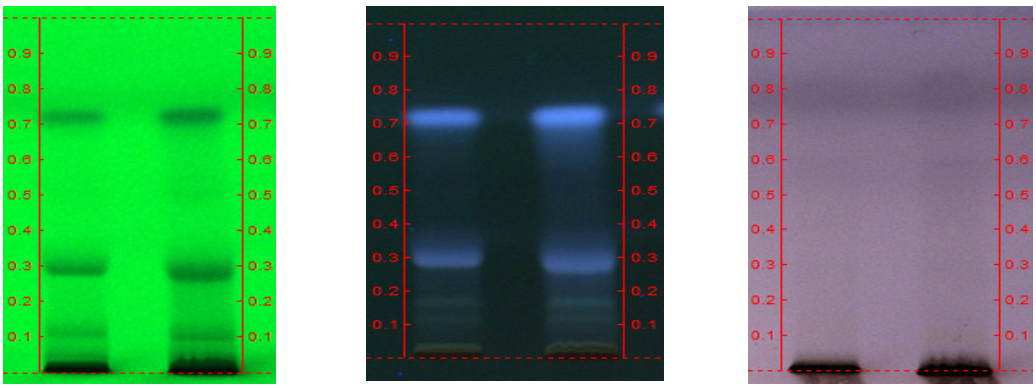
Continuation of the table

Phytochemical constituents	Hexane	Ethyl acetate	Acetone	Methanol	Distilled water
Glycosides	-	-	+	+	+
Phenols	-	+	+	+	+
Saponins	-	+	+	+	+
Steroids	-	-	-	-	-
Tannins	-	+	+	+	+
Terpenoids	-	-	+	+	+
Quinones	-	-	-	+	-
Reducing sugar	-	-	-	+	-
Carbohydrates	-	-	-	+	-
Iridoids	-	-	-	-	-
Note: "+"indicates the presence of phytochemical; "-" indicates the absence of phytochemical					

Alkaloids exhibit a wide range of medicinal properties and have various applications, including antiparasitic, antiplasmodial, anticorrosive, antioxidative, antibacterial, anti-HIV, and insecticidal activities [11]. Phenolics and flavonoids are also well-documented for their extensive pharmacological activities [12]. Additionally, terpenoids, a class of isoprenoids, are known to possess numerous biological activities [13]. The detection of these compounds in different plant parts highlights the potential for further phytochemical research.

To determine the number of compounds, HPTLC analysis was performed on the methanolic extract of the bark. Since most plant constituents react with vanillin–sulphuric acid to produce coloured zones, this spray reagent was used to detect the presence of phytochemicals in the *S. stocksii* methanolic bark extracts. The R_f values and colour reactions of the compounds were compared. Identical colours and R_f values under the same experimental conditions indicated the presence of similar compounds. The chromatograms of *Syzygium stocksii* bark extract at UV 254 nm and 366 nm revealed that all sample constituents were clearly separated without any tailing and diffuseness. The HPTLC chromatogram of ethanolic extract recorded at 254 nm, 366 nm and after derivatization with vanillin – sulphuric acid at 575 nm was depicted on Figure 1.

The HPTLC fingerprint profiles, R_f values, and area obtained for extracts after scanning at UV 254 nm, 366 nm, and after derivatization with vanillin sulphuric acid are given in Figures 2, 3 and 4 and Tables 5, 6 and 7, respectively.



Under UV short | Under UV long | Under white light after derivatisation

Figure 1 – HPTLC chromatogram of methanolic extract of *Syzygium stocksii* bark

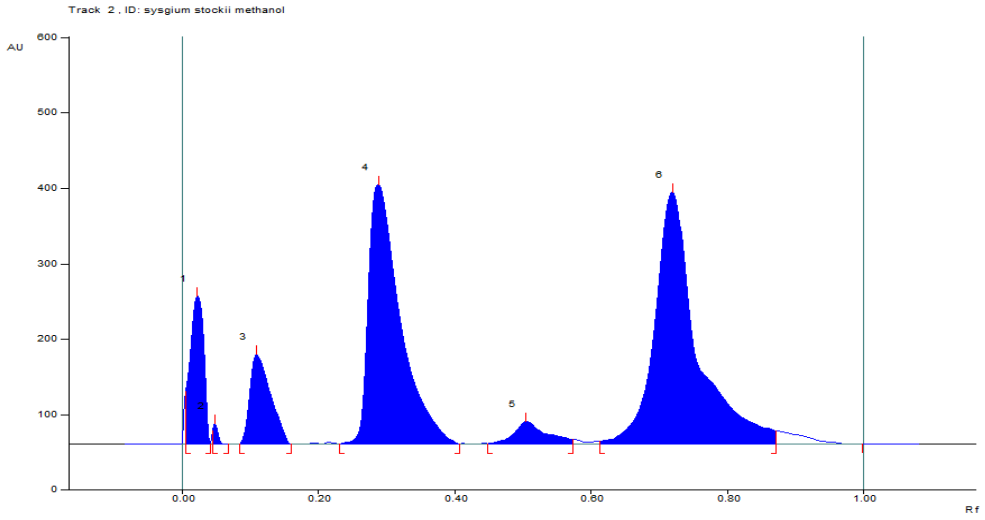


Figure 2 – HPTLC fingerprint of *Syzygium stocksii* bark at 254 nm

Table 5 – HPTLC peak table of methanolic extract of *Syzygium stocksii* at 254 nm

Peak	Start Position	Start Height	Max Position	Max height	Max %	End Position	End Height	Area	Area%
1	0.00 Rf	69.6 AU	0.02 Rf	195.8 AU	18.67%	0.04 Rf	0.4 AU	2761.0 AU	8.76%
2	0.05 Rf	16.9 AU	0.05 Rf	26.7 AU	2.55%	0.07 Rf	0.0 AU	134.3 AU	0.43%
3	0.09 Rf	0.4 AU	0.11 Rf	118.4AU	11.29%	0.16 Rf	0.0 AU	2478.8 AU	7.86%
4	0.23 Rf	0.0 AU	0.29 Rf	343.9 AU	32.78%	0.41 Rf	0.2 AU	10932.6 AU	34.67%
5	0.45 Rf	0.6 AU	0.50 Rf	30.1 AU	2.87%	0.57 Rf	5.8 AU	993.2 AU	3.15%
6	0.61 Rf	3.8AU	0.72 Rf	334.0 AU	31.84%	0.87 Rf	17.5 AU	4234.3 AU	45.14%

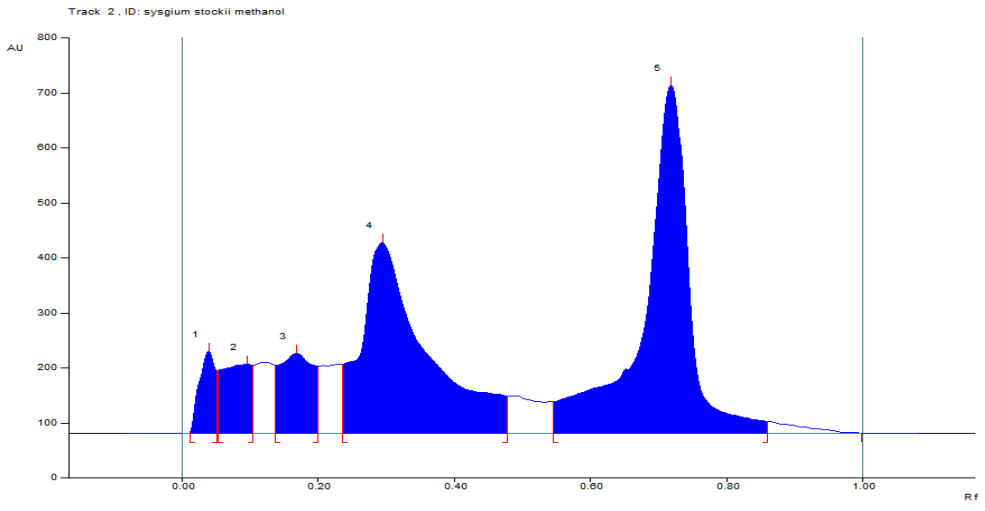


Figure 3 – HPTLC fingerprint of *Syzygium stocksii* bark at 366 nm

Table 6 – HPTLC peak table of methanolic extract of *Syzygium stocksii* at 366 nm

Peak	Start Position	Start Height	Max Position	Max height	Max%	End Position	End Height	Area	Area %
1	0.01 Rf	1.7 AU	0.04 Rf	149.4 AU	10.66%	0.05 Rf	14.0 AU	2456.9 AU	3.89%
2	0.05 Rf	114.3 AU	0.10 Rf	126.1 AU	9.00%	0.10 Rf	23.9 AU	3884.0 AU	6.14%
3	0.14 Rf	124.5 AU	0.17 Rf	145.1 AU	10.36%	0.20 Rf	22.7 AU	5034.5 AU	7.96%
4	0.24 Rf	125.9 AU	0.29 Rf	346.7AU	24.74%	0.48 Rf	67.9 AU	22909.3 AU	36.23%
5	0.55 Rf	57.5 AU	0.72 Rf	633.9 AU	45.24%	0.86 Rf	21.8 AU	28944.9 AU	45.78%

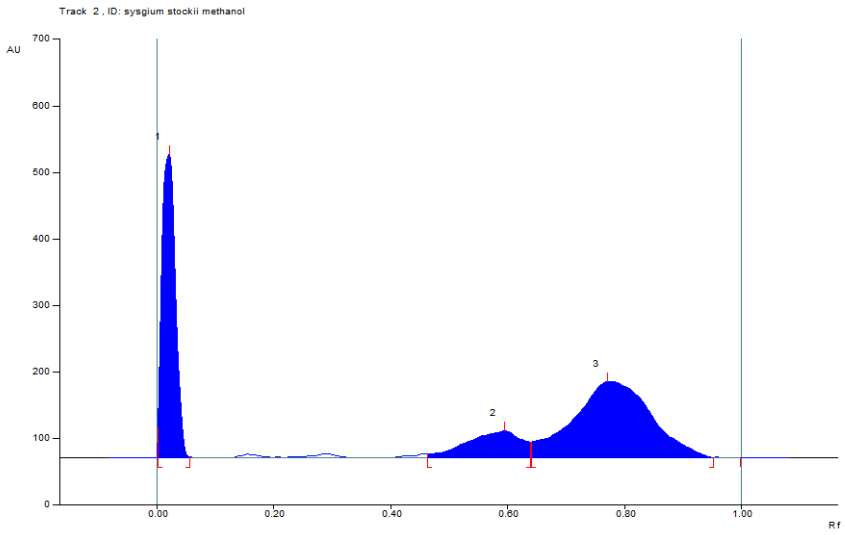


Figure 4 – HPTLC fingerprint of *Syzygium stocksii* bark at 575 nm

Table 7 – HPTLC peak table of methanolic extract of *Syzygium stocksii* at 575 nm

Peak	Start Position	Start height	Max position	Max height	Max %	End position	End height	Area	Area%
1	0.00 Rf	45.3AU	0.02 Rf	455.6AU	74.61%	0.06 Rf	1.8AU	7464.2AU	36.04%
2	0.46 Rf	5.4 AU	0.60 Rf	40.4 AU	6.62%	0.64 Rf	23.7AU	2608.2AU	12.59%
3	0.64 Rf	23.8AU	0.77 Rf	114.6AU	18.76%	0.95 Rf	0.4 AU	10639 AU	51.37%

The chromatograms observed under UV light at 254 nm and 366 nm revealed clear separation of all sample constituents. In the methanolic extract of the bark, six peaks were identified, indicating the presence of at least six different components. At 254 nm, one of the components, with an Rf value of 0.87, occupied 45.15% of the total area. After derivatization with vanillin–sulphuric acid reagent, five peaks were observed at 366 nm and three peaks at 575 nm, suggesting further differentiation of the components.

FTIR support a material's ability to absorb light by analyzing how different molecular compounds respond to an infrared beam. This interaction helps determine the composition of the material under investigation. The FTIR spectrum in this study, presented in Figure 5, reveals several distinct absorption bands. A broad band is observed in the single-bond region, while a peak at 3214.40 cm^{-1} confirms the presence of ammonium ions. In the triple-bond region, a peak at 2038.73 cm^{-1} suggests the presence of cyanide and thiocyanate ions, while

a peak at 1983.29 cm^{-1} may indicate alkynes. The strong C=O stretching at 1718.75 cm^{-1} suggests the presence of formates or unsaturated esters. The peak at 1604 cm^{-1} points to C-C stretching and N-H bending, indicating conjugated alkenes and amines.

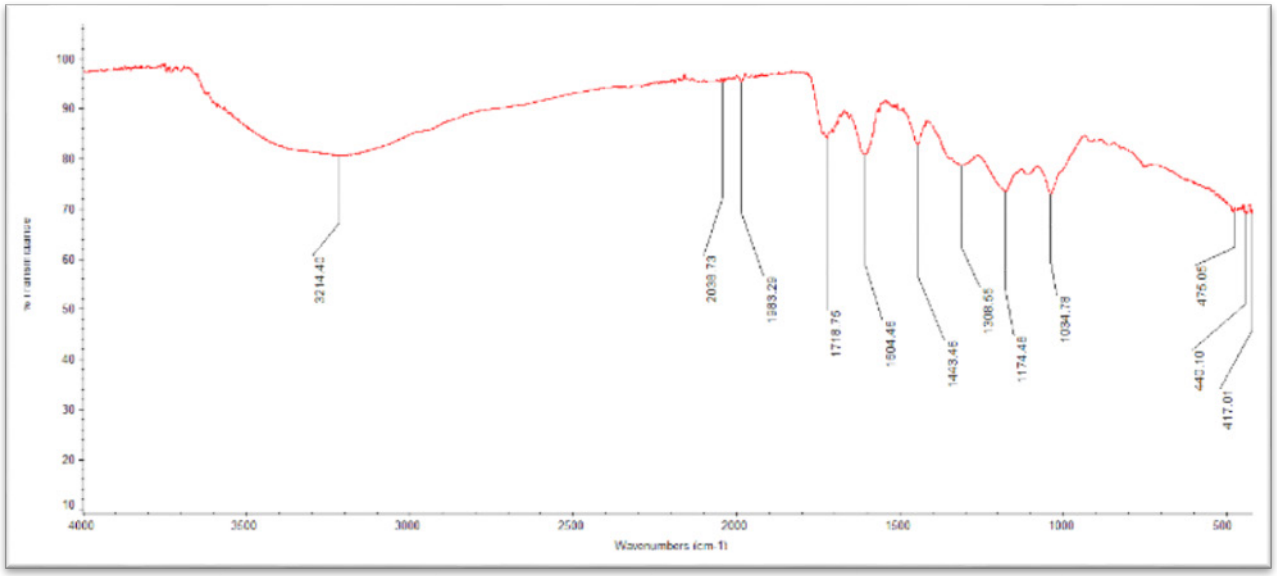


Figure 5 – FTIR Spectra of methanolic bark extract of *Syzygium stocksii* extract

Further, a peak at 1443.46 cm^{-1} suggests the presence of carbonate ions, while the peak at 1308.55 cm^{-1} corresponds to strong S=O and C-O stretching, pointing to sulphones and aromatic esters. Strong C=O stretching at 1174.48 cm^{-1} indicates esters, and the peak at 1034.78 cm^{-1} suggests the presence of S=O in sulphoxide ions. Three additional peaks in the fingerprint region, at 475.05 cm^{-1}, 440.10 cm^{-1}, and 417.01 cm^{-1}, were also observed. The fingerprint of a sample can be seen in an infrared spectrum, where the absorption peaks signify the frequencies of vibrations between the atoms that make up the substance [14]. All materials are made up of diverse combinations of atoms, hence no two compounds will have the same infrared spectrum. It can also lead to the identification of distinct type of material [15].

Conclusion

The preliminary phytochemical analysis is crucial for identifying compounds that contribute to the biopotential and traditional medicinal uses of *Syzygium stocksii*. The analyses conducted reveals the chemistry of the plant compounds in the bark of the plant.This study provides insights into the phytochemicals present in the plant, all of which play a significant role in treating various ailments. Further in-depth pharmacological and phytochemical characterization is essential to confirm its therapeutic potential.

Acknowledgment

Acknowledgments to the Head of the department of Botany, University of Kerala, and University Grants Commission (UGC) for providing Junior Research Fellowship.

Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

References

1. Pawar A. P., Patil V. K., Patil M. D., Rane A. D., Mhaiske V. M., & Tripath V. D. (2023). Distribution and phenology of *Syzygium stocksii* (Duthie) Gamble, an endangered tree species in South Konkan. *Indian Journal of Ecology,* 50(4), pp. 963-968. https://doi.org/10.55362/IJE/2023/3996.

2. Mitra S.K., Irenaeus T.K.S., Gurung M.R. and Pathak P. (2012). Taxonomy and importance of Myrtaceae. *Acta Horticulturae*, 959, pp. 23-34. https://doi.org/10.17660/ActaHortic.2012.959.2.

3. Chandra A.S., Siril E.A., Radhamany P.M. (2020) Phytochemical profile of *Syzygium caryophyllatum* (L.) Alston. *International Journal of Botany Studies*, 5, 6, pp. 599-602.

4. Aung E.E., Kristanti A.N., Aminah N.S., Takaya Y., Ramadhan R. (2020) Plant description, phytochemical constituents and bioactivities of *Syzygium genus*: A review. *Open Chemistry*, 18, 1, pp. 1256-1281. https://doi.org/10.1515/chem-2020-0175.

5. Roby T.J., Joyce J., Nair P.V. (2013) *Syzygium travancoricum* (Gamble)-a critically endangered and endemic tree from Kerala, India- threats, conservation and prediction of potential areas; with special emphasis on Myristica swamps as a prime habitat. *International Journal of Science*, 2, 6, pp. 1335-1352.

6. Evans W.C. (1997) Pharmacognosy 13th Edition. London: Balliere, Tindall, pp. 23-54.

7. Gupta R., Gabrielsen B., Ferguson F.M. (2005) Nature's medicines: traditional knowledge and intellectual property management. Case studies from the National Institutes of Health (NIH), USA. *Curr. Drug Discov. Technol*., 2, pp. 203-219.

8. Harborne J.B. (1973) Phytochemical methods. London: Chapman and Hall Ltd., pp. 49-188.

9. Harborne J.B. (1973) Phytochemical methods, a guide to modern techniques of plant analysis. London: Chapman and Hall Ltd., pp. 12-236.

10. Iyengar M.A. (1995) Study of Crude Drugs. 8th edition, India, Manipal: Manipal Power Press.

11. Kurek Joanna. (2019) Introductory Chapter of Alkaloids – Their Importance in Nature and for Human Life, ed. by Joanna Kurek. IntechOpen, 2019. https://doi.org/10.5772/intechopen.85400.

12. Tungmunnithum D., Thongboonyou A., Pholboon A., Yangsabai A. (2018) Flavonoids and Other Phenolic Compounds from Medicinal Plants for Pharmaceutical and Medical Aspects: AnOverview. *Medicines (Basel,Switzerland)*, 5, 3, p. 93. https://doi.org/10.3390/medicines5030093.

13. Perveen S. (2018) "Introductory Chapter: Terpenes and Terpenoids". Terpenes and Terpenoids, edited by Shagufta Perveen, Areej Al-Taweel. IntechOpen. https://doi.org/10.5772/intechopen.79683.

14. Nisreen A., Eltahir M. A., Siddig A. A., Siddig, Hajir Adam (2013) Structure and physical properties of flowering plants of the genus hibiscus, *Sudan Medical Monitor*, 8, 3, pp. 135-139.

15. Siddig M. A., Abdelgadir A. E., Elbadawi A. A., Mustafa E. M., & Mussa, A. A. (2015) Structural characterization and physical properties of Syzygium cumini flowering plant. *International Journal of Innovative Research in Science, Engineering and Technology,* 4, 5, pp. 2694-2700, https://doi.org/10.15680/IJIRSET.2015.0405006.

Information about authors:

Adheena Elza Johns – (corresponding author) – M.Sc., Assistant Professor, Department of Botany, St.Thomas College, Kozhencherry, Kerala, India, e-mail: adheenajohns@gmail.com

Radhamany Puthuparambil Madhavan – Dr., Emeritus Professor, Department of Botany, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, India, e-mail: radhamany_m@rediffmail.com