Phytochemical and Fourier Transform Infrared Spectroscopy Analysis of *Rivea hypocrateriformis* (Desr.) Choisy.

***Iswarya S and **Mary Kensa V**

*Research Scholar (Full time), Reg: No: 20113152262022,

Abishehapatti, M.S.University, Tirunelveli.

**PG Research Centre of Botany, S.T. Hindu College, Nagercoil.

ABSTRACT

Medicinal plants play a significant part in natural wealth.Phytochemicals are non-nutritive chemical compounds that occur naturally on plants and have diverse protective properties.The phytochemical analysis shows the presence of alkaloids, saponins, phenol, flavonoids, tannins, steroids and proteins in ethanol, ethyl acetate, chloroform, butanol and aqueous extracts of *Rivea hypocrateriformis* (Desr.) Choisy. FTIR perhaps the most powerful tool for identifying the types of chemical bonds / functional groups present in the phytochemicals. FTIR analysis in the whole dry powdered material in the ethanol extracts of *R.hypocrateriformis*. provides the various phytochemicals having functional groups such as hydroxyl compound, methyl group, cyclo alkane, carbonyl compound, sulphur compound, alkyl ketone, aminoacids, sulphones compound, halogen compound and alkyl halides. The wavelength of light absorbed is the salient features of the chemical bonds seen in the annotated spectrum.The results confirm the fact that these plant posses important bioactive constituents useful for our health, so further scientific investigation is needed. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and also bioactivity.

KEY WORDS:

Acetone,bio-efficacy, ethyl acetate,*Rivea hypocrateriformis*and spectrum.

INTRODUCTION

Medicinal plant research includes much more than the discovery of new drugs They are the great source of herbal drugs.The connection between human wellbeing and plants exist from fossils history around 60,000 years prior. Around 215,000 to 500,000 types of higher plants remain alive on earth. Yet, just 6% of plants are being utilized for the organic action (Farnsworth, 1988; Abbasi*et al.,* 2018). Nature has given the tremendous variety of restorative plants and strong bioactive constituents for mankind as long numerous years; anyway plants are the fortunes for the wellspring of prescriptions for the essential medical services framework (Hamsalakshmi, and Akassh, 2020).

In flow years, Indian restorative plants have been explored by analysis for pharmacological movement. The distinctive phyto-constituents present in restorative plants are flavonoid, alkaloid, phenol and tannins, carboxylic acids, terpenes and amino acids and inorganic acids. These phyto-constituents give explicit peculiarity and properties to plants. (Kalaichelvi and Dhivya, 2017).

The use of FTIR Spectroscopy demonstrates thatthis technique is a valuable group belonging to tissue or plant components as well as for complex biological materials to identify the chemical constituents and elucidate the structural compounds for identifying medicines in many countries (Helm *et al*., 1999; Janakiraman *et al.*, 2011).

MATERIALS AND METHODS

Source of the plant sample:

Rivea hypocrateriformis (Desr.)Choisy. was collected from Kottaram. It is a village located in Kanyakumari district of Tamil Nadu. This village is used to be a resting place for the Travancore Maharajas. It is very close to Kanyakumari, Vattakottai and Marunthuvazh Malai. It is a mesmerizing beautiful place.

Preparation of whole plant dry powder of selected species for Phytochemical analysis:

The selected species were collected and shade dried, powered and 2 g of air dried powder of the sample was extracted with 50 ml of solvents such as Ethanol, Ethyl acetate, Chloroform, Butanol and Aqueoususing Soxhlet apparatus for 8 hours. The extracts were filtered and filtrates were concentrated under reduced pressure at 40^{o} C using a rotary flash evaporator and it was then transferred to glass vials and kept at 4°C before use.

Preparation of whole plant dry powder of selected species for FTIR analysis:

The selected species were collected and dried separately at room temperature ($25\pm2^{o}_{C}$) and powdered in mechanical grinder. 150 ml of solvent (ethanol) were added and kept for three days. The extract was filtered using Whatman No.1 filter paper and the supernatant was collected. The residue was again extracted two times (with 3 days of intervals for each extraction) and supernatants were collected. The supernatants were pooled and evaporated (at room temperature, $28 \pm 1^{o}_{C}$) until the volume was reduced to 150 ml and were stored in air tight bottles for subsequent analysis.

Qualitative Phyto-Chemical Analysis (Harbone, 1973**)**

The various solvent extracts of *Rivea hypocrateriformis*wassubjected to qualitative chemical investigation.

Details of various tests

The following procedures were adopted to test for the presence of various chemical constituents in the extracts.

Tests for alkaloids

a. **Mayer's test:** Test solution treated with Mayer's reagent (Potassium mercuric iodide) gives cream coloured precipitate.

b. **Wagner's test:** The acidic solution treated with Wagner's reagent (Iodine in potassium iodide) gives brown precipitate.

c. **Hager's test:** The acidic solution with Hager's reagent (Saturated picric acid solution) gives yellow precipitate.

d. **Dragendorff's test:** The acidic solution with Dragendorff's reagent (potassium bismuth iodide) shows reddish brown precipitate.

Test for carbohydrates

a. **Molisch's test:** Test solution with few drops of Molisch's reagent and 2 mL of concentrated Sulphuric acid is added slowly from the sides of the test tube shows a purple ring at the junction of two liquids.

b. **Barfoed's test:** Test solution treated with Barfoed's reagent on boiling on a water bath shows brick red precipitate.

c. **Benedict's test:** Test solution treated with Benedict's reagent and boiling on a water bath shows reddish brown precipitate.

Test for saponins

a. **Foam test:** Saponins when mixed with water and shaken shows the formation of foam, which is stable at least for 15 minutes.

b. **Haemolysis test:** 2 mL of 18% sodium chloride in two tubes was taken. To one test tube distilled water is added and to other test tube 2 mL of filtrate and then few drops of blood is added to both the test tubes. Mixed and observed for haemolysis under microscope.

Test for phenols

Ferric chloride test: Two milliliters of 5% solution of $FeCl_3$ were added to 1 ml crude extracts. A black or blue-green colour indicated the presence of phenols.

Test for flavanoids

a. **Ferric chloride test:** Test solution with few drops of ferric chloride solution shows intense green colour.

b. **Shinoda test:** Test solution with few fragments of magnesium ribbon and concentrated Hydrochloric acid shows pink to magenta red colour.

c. **Zinc-Hydrochloric acid-reduction test:** Test solution with zinc dust and few drops of HCL shows magenta red colour.

d. **Alkaline reagent test:** Test solution when treated with sodium hydroxide solution shows increase in the intensity of yellow colour which becomes colourless on addition of few drops of dilute acid.

e. **Lead acetate solution test:** Test solution with few drops of lead acetate solution (10%) gives yellow precipitate.

Tests for tannins

a. **Ferric-chloride test:** Test solution with few drops of ferric chloride solution gives dark colour.

b. **Gelatin test:** Test solution treated with gelatin solution gives white precipitate.

Steroids

Five milliliters of chloroform and 5 ml of H_2O_4 were added to 500 µl of the prepared plant extracts. The presence of steroids was indicated by a colour change from violet to blue or green or a ring of blue/green or if the upper layer turns red and the sulphuric layer was yellow with a green fluorescence.

Tests for protein

a. **Million's test:** Test solution treated with million's reagent and heated on a water bath, protein is stained yellow on warming.

b. **Xanthoproteic test:** Test solution treated with conc. nitric acid and on boiling gives yellow precipitate.

c. **Biuret test:** Test solutions treated with 40% sodium hydroxide and dilute copper sulphate solution gives blue colour.

d. **Ninhydrin test:** Test solution treated with ninhydrin reagent gives blue colour.

Test for glycosides

a. **Baljet's test:** The test solution treated with sodium picrate givesyellow to orange colour.

b. **Keller-Killiani test:** The test solution with few drops glacial acetic acid in 2 mL of ferric chloride solution and concentrated sulphuric acid is added from the sides of test tube which shows the separation between two layers, lower layer shows reddish brown and upper layer turns bluish green.

c. **Raymond's test:** Test solution treated with dinitrobenzene in hot methanolic alkali gives violet colour.

d. **Bromine water test:** Test solution dissolved in Bromine water gives yellow precipitate.

e. **Legal's test:** Test solution when treated with pyridine (made alkaline by adding sodium nitroprusside solution) gives pink to red colour.

Fourier Transform Infrared Spectrophotometer (FTIR):

Fourier Transform Infrared Spectrometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bond / functional groups present in the phytochemicals. The wavelength of light absorbed is salient feature of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bond in the compound can be determined. Dried powder of ethanol extract of *Rivea hypocrateriformis* was used for FTIR analysis. 10 mg of dried extract powder was encapsulated in 100 mg of KBr pellet, in order to prepare translucent sample discs. The powdered sample of each 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}. All the analysis was repeated thrice and mean ± SD was recorded.

RESULT AND DISCUSSION

Table : 1 Phytochemical analysis of *Rivea hypocrateriformis* (Desr.) Choisy. using various extracts :

S.No	Tests	Ethanol	Ethyl acetate	Chloroform	Butanol	Aqueous
1	Alkaloid	+	+	-	+	-
2	Carbohydrates	-	-	-	-	-
3	Saponins	+	-	+	+	-
4	Phenol	+	+	+	-	+
5	Flavonoids	+	-	-	-	+

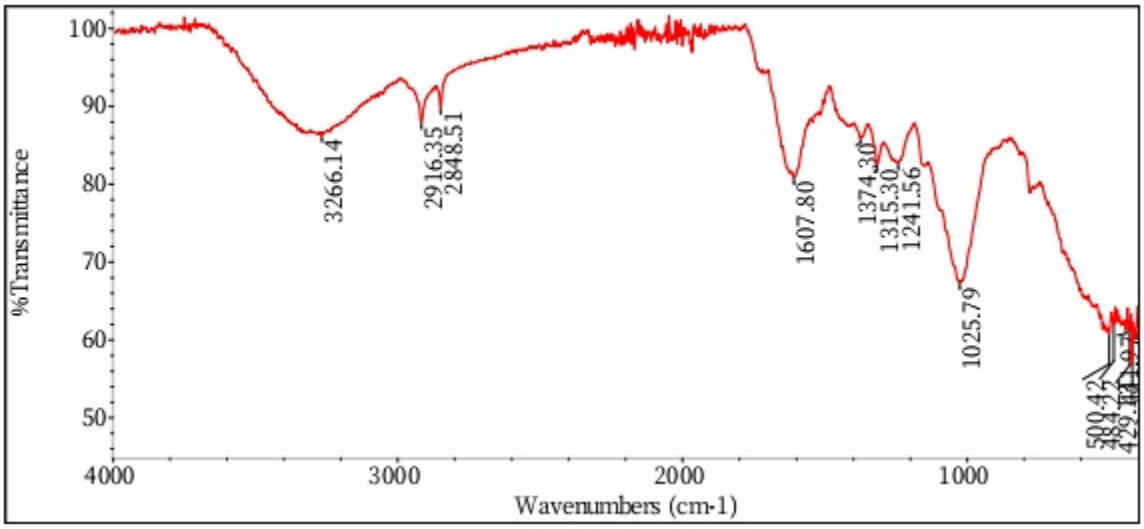
6	Tannins	+	+	-	-	+
7	Steroids	-	+	+	-	-
8	Protein	+	+	+	+	-
9	Glycosides	-	-	-	-	-

+ = Present; - = Absent

Table 2: FTIR analysis of the ethanolic extract of *Rivea hypocrateriformis* (Desr.) Choisy.

S.No	Frequency Range (cm^{-1})	Functional Group
1	3266.14	Hydroxyl Compound
2	2916.35	Methyl Group
3	2848.51	Cyclo alkane
4	1607.80	Carbonyl Compound
5	1374.30	Sulphur Compound
6	1315.30	Alkyl ketone
7	241.56	Amino acid
8	1025.79	Sulphones Compound
9	500.42	Halogen Compound
10	441.97	Alkyl halides

Figure 1: Chromatogram for the FTIR analysis in the ethanol extract of *Rivea hypocrateriformis* (Desr.) Choisy:



The phytochemical analysis of the ethanol, ethyl acetate, chloroform, butanol and aqueous extracts of *R. hypocrateriformis*shows the presence of alkaloids, saponins, phenol, flavonoids, tannins, steroids and proteins which is depicted in the table 1. Thus, the preliminary screening tests might be helpful in the identification of bioactive principle and may prompt to the drug discovery and improvement.

The ethanol extract of *R. hypocrateriformis* showsFTIR Spectrum which is used for identifying the functional group of active components based on the peak value in the region of infra red radiation and the FTIR peak values and functional groups were represented in table 2& figure 1. The ethanolic extract of *R.hypocrateriformis* is passed into the FTIR Spectroscopy and the functional groups of the components are separated based on the peak ratio. The results of FTIR analysis confirm the presence offunctional groups such as hydroxyl compound, methyl group, cyclo alkane, carbonyl compound, sulphur compound,alkyl ketone, amino acids, sulphones compound, halogen compound and alkyl halide showing the frequency range (cm^{-1}) such as 3266.14, 2916.35, 2848.51, 1607.80, 1374.30, 1315.30, 1241.56, 1025.79, 500.42 and

484.22cm-¹ respectively and are represented in the table2 and figure1. Based on the functional group analysis, it is evident that *R. hypocrateriformis* plant does not consist of any toxic compound.

Phytoconstituents like saponins, phenolic mixtures and glycosides have been accounted for to restrain bacterial development and to be defensive of plants against bacterial diseases (Menaga and Ayyasamy, 2012).Fragrant plants with rich phenolic compounds has great medicinalqualities which assists with treating different illnesses (Vinoth Kumar*et al.,* 2017).The medicinalemployments of alkaloids found in the plant leaves have been known for quite a long time, and among them, cytotoxicity is one of their biologicalproperties (Jacques Britto & Kesavi Durairaj, 2020).

FTIR analysis of *Rivina humilis* confirmed the presence of 9 peaks (primary/ secondary amines) at peak 3288.04 (Kavitha *et al.,* 2019). The results of functional group analysis using FTIR revealed the existence of various characteristic functional groups in leaves, stem, flower and fruit of *Atylosa albicans* and *Tephrosia tinctoria* (Komal *et al.,* 2011). FTIR analysis for functional groups revealed the presence of various characteristic functional groups in both the samples (dried plant powder and methanol extract) of *Cleome gynandra* (Deepashree *et al.*, 2012). Fourier transform infrared spectroscopy is a high resolution analytical technique to identify the chemical constituents and elucidate the structure of compounds (Hashimoto and Kameoka, 2008, Hussian *et al.,* 2009).FTIR combines withpartial least square and principal component regression has been used for quantification of circuminoid in extracts of *Curcuma longa* (Rohman *et al*., 2015) and *Curcuma xanthorrhiza* (Lestari *et al.,* 2017). FTIR analysis of *Myristica dactyloids* fruit extract shows the presence of different functional groups such as carboxylic acid, aromatics, alkanes, alcohols, phenols, aliphatic amines, alkenes and amine

groups (Rajiv *et al.*, 2017). FTIR analysis of the methanol extract of *Ceropegia juncea* given the major peak observed was at wavenumber 3354.08 cm-¹ that indicates the presence of O-H alcohol functional group (Visveshwari *et al.,* 2017). FTIR analysis of some medicinal plants, *Celotropis gigantean, Tylophora pauciflora, Caralluma geniculata and Caralluma nilagiriana*reported 28 components with different chemical structure (Kalimuthu and Prabakaran, 2013). FTIR analysis of the methanol extract of *Eichhornia crassipes* suggests the presence of aromatic benzene, aliphatic amines, alkanes, amines ether, carboxylic acid and phenols (Geethu *et al*., 2014). FTIR analysis of methanolic stem extract of *Faidherbia albida* confirmed the presence of primary amine, alcohol, alkyl halide, alkane and aldehyde (Maitera and Chukkol, 2016). FTIR spectrum in whole plant extract of *Aerva lanata* confirmed the presence of alkyl group, aldehyde, ester and anhydrides (Manickam *et al.,* 2014).

CONCLUSION

Phytochemicals in greenery nourishment had extraordinary arrangements of fascination. Qualitative examination of phytochemical was all the more fascinating territory and furthermore significant use of biomedical in pharmaceutical businesses. The results for phytochemical and FTIR analysis of *Rivea hypocrateriformis*confirms the fact that these plant posses important bioactive constituents useful for our health, so further scientific investigation is needed. Further work will emphasize the isolation and characterization of active principles responsible for bio-efficacy and also bioactivity.

REFERENCES

1. **Abbasi, W. M., Ahmad, S., Perveen, S. and Rehman, T. (2018).** Preliminary phytochemical analysis and in vivo evaluation of antipyretic effects of hydro-methanolic

extract of *Cleome scaposa* leaves. *Journal of traditional and complementary medicine*, ***8*(1):** 147-149.

2. **Deepashree, C..Lingegowda, Komal kumar, J., Devi Prasad, A.G., Mahsa Zarei and Shubha Gopal. 2012**. FTIR Spectroscopic studies on *Cleome gynandra* – comparative analysis of functional group before and after extraction. *Romanian Journal of Biophysics.***22(3):** 137-143**.**
3. **Geethu, M.G., Suchithra, P.S., Kavitha, C.H., Aswathy, J.M., Dinesh Babu and Murugan, K. 2014.** Fourier transform infrared spectroscopy analysis of different solvent extracts of water hyacinth (*Eichhornia crassipes*) an allopathic approach. *World Journal of Pharmacy and Pharmaceutical Sciences.***3(6):** 1256-1266.
4. **Hamsalakshmi, S. J. and Akassh, M. (2020).** Phytochemical Analysis and In-vitro Antioxidant Activity of Aerial Parts of *Trichodesmaindicum*. *International journal of research in pharmaceutical sciences*. **11(2):**1386-1393.
5. **Hashimoto, A. and Kameoka, T. 2008.** Applications of infrared spectroscopy to biochemical, food and agricultural processes. *Applied Spectroscopy Reviews.***43:** 416-451.
6. **Helm, D., Labischinski, H., Schallehn, G. and Naumann, D. 1991.** Classification and identification of bacteria by Fourier transform infrared spectroscopy. *J. Gen. Microbiol.***137:** 69-79.
7. **Hussian, K., Ismail, Z., Sadikun, A. and Ibrahim, P. 2009.** Evaluation of metabolic changes in fruit of *Piper armentosum* in various seasons by metabolomics using Fourier Transform Infrared Spectroscopy. *International Journal of Pharmaceutical and Clinical Research.***1(2):** 68-71.
8. **Jacques Britto N. and Kesavi Durairaj. (2020).** Phytochemical profile and medicinal potentials of *Lannea Coromandelica* stem. *International Journal of Research in Pharmaceutical Sciences*, **11(3):** 3465-3472.

9. **Janakiraman, N., Sahaya Sathish, S. and Johnson, M. 2011.** UV-VIS and FTIR spectroscopic studies on *Peristrophe bicalyculata*(Retz.)Nees. Asian Journal of Pharmaceutical and Clinical Research. **4(4):** 125-129.
10. **Kalaichelvi, K and Dhivya, S.M. (2017).** Screening of phytoconstituents, UV-VIS Spectrum and FTIR analysis of *Micrococcamercurialis* (L.) Benth. *International Journal of Herbal Medicine*, **5(6):** 40-44.
11. **Kalimuthu, K and Prabakaran, R.** 2013. Preliminary phytochemical screening, GC-MS analysis of methanol extract of *Cerppegia pusilla.International Journal of Research in Applied, Natural and Social science.* **1(3):** 49-58.
12. **Kavitha, A., Mary Kensa, V., Neelamegam, R. and Salom Gnana Thanga, V. 2019.** Fourier Transform Infrared Spectroscopy analysis of ethanolic extract of *Rivinia Humilis L. Pramana Research Journal*. **9(6):** 585-591.
13. **Komal Kumar, J. and Devi Prasad, A.G. 2011.** Identification and comparison of biomolecules in medicinal plants of *Tephrosia tinctoria* and *Atylosia albicans* by using FTIR. *Romanian Journal of Biophysics.***21(1):** 63-71.
14. **Lestari, H.P., Martono, S., Wulandari, R. and Rohman, A. 2017.** Simultaneous analysis of Curcumin and demethoxycurcumin in *Curcuma xanthorrhiza* using FTIR spectroscopy and chemometrics. *Int Food Res J.* **24(5):** 2097-2101.
15. **Maitera, O.N and Chukkol, I.B. 2016.** Phytochemical and Fourier transform infrared spectroscopy analysis of *Faidherbia albida* as a preservative agent. *World Journal of Research and Review*.**3(3):** 25-29.

16. **Manickam Murugan and Veerabahu Ramasamy Mohan. 2014.** Phytochemical, FTIR and anti-bacterial activity of whole plant extract of *Aerva lanata. Journal of Medicinal Plant Studies.***2(2):** 51-57.
17. **Menaga, D. and Ayyasamy, P.M. (2012).** Effect of Horse Gram on the cultivation of *Pleurotus florida* Mushroom and their Phytochemical Analysis and Antimicrobial Activity. *International Journal of Research in Pharmaceutical Sciences*, **3(1):** 140-145.
18. **Rajiv, P., Deepa, A., Vanathi, P. and Vidhya, D. 2017.** Screening for phytochemicals and FTIR analysis of *Myristica dactyloids* fruit extracts. *International Journal of Pharmacy and Pharmaceutical Sciences.***9(1):** 315-318.
19. **Rohman, A., Sudjadi, Ramadhani, D. and Nugroho, A. 2015.** Analysis of Curcumin in *Curcuma longa* and *Curcuma xanthorrhiza* using FTIR Spectroscopy and chemometrics. *Res. J. Med. Plant.***9(4):** 179-186.
20. **Vinoth Kumar, S., Gopal, V. and Devanna, N. (2017).** Antidiabetic activity of ethanolic extract of *Rhynchosia suaveolens* (L.F.) DC. in Streptazotocin induced diabetic rats. *International Journal of Research in Pharmaceutical Sciences*, **8(4):** 596-602.
21. **Visveshwari, M., Subbaiyan, B and Thangapandian, V. 2017.** Phytochemical analysis, antimicrobial activity, FTIR and GC-MS analysis of *Ceropegia Juncea* Roxb. *International Journal of Pharmacognosy and Phytochemical Research.***9(7):** 914-920. Farnsworth, N. R. (1988). Screening plants for new medicines. *Biodiversity*, *15*(3), 81-99.