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SPECTROSCOPIC STUDY ON METHANOLIC EXTRACT OF *CITRUS RETICULATA* BLANCO FRUIT PEEL

Jerlin Showmya J[1], Pradeepa M[2] and Geetha N*

[1]Research Scholar,[*] Professor and Head, Department of Biotechnology, Mother Teresa Women's University, Kodaikanal, Tamil Nadu-624101.

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***Correspondence for Author**
Geetha. N
Research Scholar,
Professor and Head,
Department of Biotechnology, Mother Teresa Women's University, Kodaikanal, Tamil Nadu-624101.

ABSTRACT

In the present study characterization of bioactive constituents present in different methanolic peel extract of *Citrus reticulata* Blanco using UV-VIS, FTIR and GC-MS. The UV-VIS profile showed different peaks ranging from 100-500 nm with different absorption respectively. The FTIR spectrum confirmed the presence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, carboxylic acids, aromatics, nitro compounds and amines in methanolic extract. The results of the GC-MS analysis provide different peaks determining the presence of 23 phytochemical compounds with different therapeutic activities. Hence, this study offers a base of using *Citrus reticulata* peel as herbal alternative for various medicinal purposes.

Keywords: Citrus reticulata, phytoconstituents, UV-Vis, FT-IR and GC-MS.

INTRODUCTION

Medicinal plants are the richest bio-resources of drugs for traditional systems of medicine. Knowledge of the chemical constituents of plant is helpful in the discovery of therapeutic agents. It is well known that the medicinal materials comprises hundreds of components and produce their curative effects. The most important bioactive constituents of the plants are alkaloids, flavonoids, tannins, terpenes, amino acid, phenolic compounds, carboxylic acid and inorganic acids (Satapathy A.K *et al.*, 2009).

Phytoconstituents confer specific characteristics and properties to the plants, pure compounds or as standardized plant extracts provide unlimited opportunities for new drugs (Parekh J &

Chanda V., 2007). In order to promote the use of medicinal plants as potential source, it is important to thoroughly investigate their structural composition and activity and thus validate their uses (Nair R & Chanda S., 2006). It has been shown that *in-vitro* characterization methods could provide the needed preliminary observations to select crude plant extracts with potentially useful properties for further chemical and pharmacological investigations (Mathekaga A.D & Meyer J.J.M., 1998).

A variety of techniques can be used to determine and estimate the presence of such phytoconstituents in medicinal plants. Analysis of small amount of chemicals has become effective, easier and in-expensive owing to the development of hyphenated techniques such as UV-Vis, FT-IR and GC-MS spectroscopic techniques, which can identify pure plant compounds. Chromatography and spectroscopic techniques are the most useful and popular tools used recently for this purpose (Grube M *et al.*, 2008).

Citrus plants belong to the family Rutaceae. The most important commercially citrus cultivation in India is the Mandarian- *Citrus reticulata*. Currently there is much biomedical interest in citrus fruits and peels because of their greater consumption and it appears to be associated with low risk of cancer, obesity, cardiovascular and other diseases. Studies conducted so far on several fruit peel have shown that peels are the major sources of natural antioxidants and other phytochemical compound (Jerlin Showmya J *et al*., 2014) Since C*itrus reticulata* eel has been focussed.

Peel represents a class of interesting source of organic compounds. There is no detailed systematic documentation of presence and type of phytochemicals in *Citrus reticulata* peel. Hence, the present study aimed at an evaluation of the phytochemical compounds present in the methanolic extract by means of UV-Vis, FT-IR and GC-MS spectroscopic techniques.

MATERIALS AND METHODS

Collection and preparation of plant material

Citrus reticulata (Kodai orange) peel was collected from local farmers in Kodaikanal. Peel was removed from the fruit and washed thoroughly in running tap water to remove the dust particles. The washed peel was dried in hot air oven at a temperature of 30° – 35° C. The dried was grounded using mixer-grinder and the powder was subjected to Soxhlet extraction using methanol as solvent. The residual extracts were evaporated to dryness and stored in refrigerator for further analysis.

Spectroscopic analysis

Ultraviolet visible absorption (UV)

The ME of *C. reticulata* peel was analyzed in UV-Visible range between 200-800 nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu). This method is useful for analyzing organic compounds.

IR spectroscopy

Infrared spectroscopy is one of the powerful analytical techniques which offer the possibility of chemical identification. The technique is based on the simple fact that substance shows selective absorption in infrared region. After absorption of IR radiations, the molecules vibrate, giving rise to absorption spectrum. It is an excellent method for the qualitative analysis because except optical isomers, the spectrum of compound is unique. It is most useful for the identification of purity and structural details. This method is useful in the field of natural products, forensic chemistry and in industrial analysis of competitive products. The IR spectra of ME of *C. reticulata* peel were scanned on FT-IR-Shimadzu-8400 over the frequency range from 4000-400 cm-1.

GC-MS Spectroscopy

GC-MS analysis of ME of *C. reticulata* peel was performed using GC-MS instrument (GCMS-QP-2010) equipped with glass column SGE BPX5 and capillary dimension 30 m x 0.25 mm x 0.25 µ. The oven temperature was programmed from 80-260 °C. Inlet and interface temperature were 250 °C and 200 °C respectively. Carrier gas was helium at a flow rate of 1.0 ml/min. Ion source temperatures were maintained at 200 °C and spectra were measured.

RESULTS & DISCUSSION

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. The UV-VIS profile of methanolic extract of *Citrus reticulata* peel was taken at 100-500nm wavelength due to the sharpness of the peaks and proper baseline (Sahaya S *et el*., 2012). The profile showed the peaks at 223.5nm, 258nm, 284nm, 303nm and 326.5nm with the absorbance of 5.086, 2.67, 3.489, 3.239 and 3.863 respectively (Fig-1 and Table-1).

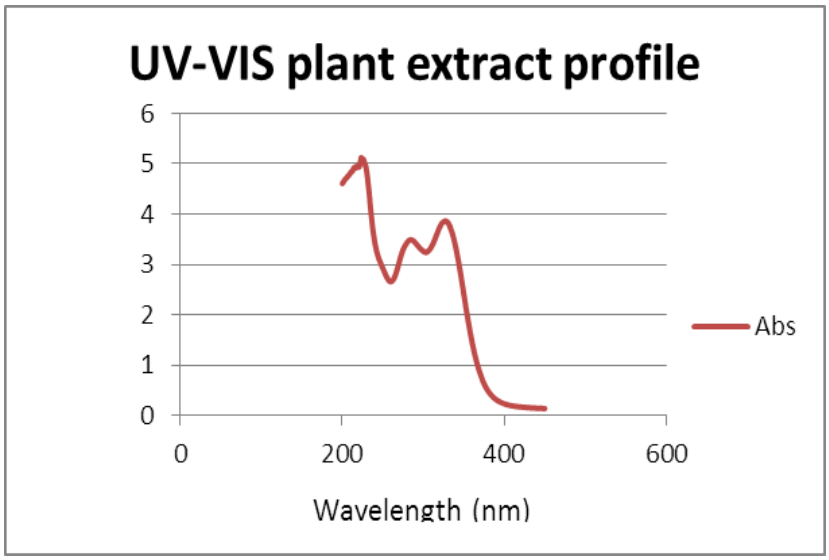

Fig: 1 UV-VIS spectrum of methanolic extract of Citrus reticulata peel

Table:1 UV-VIS peak values of methanolic extract of *Citrus reticulata* peel

S.No	Wavelength (nm)	Absorption
1	223.5	5.086
2	258	2.67
3	284	3.489
4	303	3.239
5	326.5	3.863

The FTIR spectrum was used to identify the functional groups of the active compounds based on the peak value in the region of Infrared radiation. When the plant extract was passed into the FTIR, the functional groups of the components were separated based on its peaks ratio. The results of FTIR peak value and functional groups were represented in (Fig 2 & Table 2).The analysis of IR spectra of the methanolic extract of *Citrus reticulata* peel is used to find out the various functional groups present (Mamata S *et al*., 2012). Vibrational bands of primarily important of the methanolic extract were observed around 3411.76 cm^{-1} to 628.18cm^{-1}. Infrared spectroscopy is one of the powerful analytical techniques which offer the possibility of chemical identification. The technique is based on the simple fact that chemical substance shows selective absorption in infrared region. After absorption oFT- IR radiations, the molecules vibrate, giving rise to absorption spectrum. It is an excellent method for the qualitative analysis because except optical isomers, the spectrum of compound is unique. It is most useful for the identification of purity and gross structural details. This method is useful in the field of natural products, forensic chemistry and in industrial analysis of competitive products. The IR spectra of ME of *C. reticulata* peel was scanned on FT-IR-Shimadzu-8400 over the frequency range from 4000-400 cm-1.

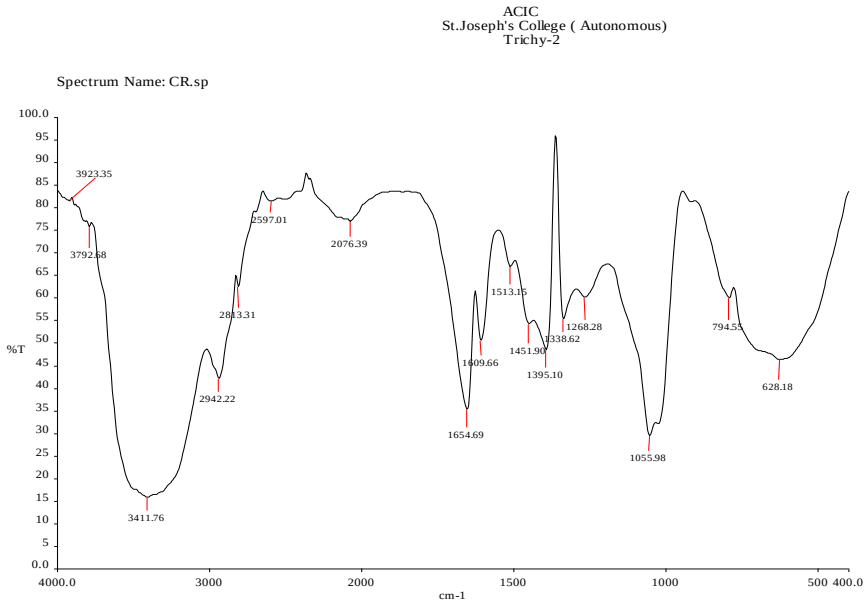

Fig :2 FTIR spectrum of methanolic extract of *Citrus reticulata* peel

Table :2 FTIR peak value of methanolic extract of *Citrus reticulata* peel

S.NO	Peak value	Functional group
1	3923.35	Unknown
2	3792.68	Unknown
3	3411.76	Primary amines
4	2942.22	Alkanes
5	2813.31	Amines (N-methyl)
6	2597.01	Organo-silicon compounds
7	2076.36	Deuterated amines
8	1654.69	Nitrites
9	1609.66	Amino acids
10	1513.15	Aromatic nitro compounds
11	1451.90	Nitrosamines
12	1395.10	Alkanes
13	1338.62	Alkanes
14	1268.28	Cong. ether
15	1055.98	Sulfoxides
16	794.55	Unknown
17	628.18	Alkynes

In the present study, GC-MS analysis of the methanolic extract of *Citrus reticular* peel revealed that the alcoholic extract is mainly composed of oxygenated hydrocarbons and predominantly phenolic hydrocarbons. These phtochemicals are responsible for various pharmacological actions like antimicrobial, anti-oxidant,anti-cancer and anti-inflammation

activities. This study is only a preliminary study of the occurrence of certain properties of extract an in-depth study will provide a good concrete base for all the biochemical and phytochemical functions mentioned above. New scientific strategies for the evaluation of natural products with specific biological activities require the implementation of large screening process (Nezhadali A *et al*., 2010 & Sathyaprabha *et al*., 2011) Interpretation on mass spectrum GC-MS was conducted using the data base of National Institute Standard and technology (NIST) having more than 62 patterns. The spectrum of unknown compounds was compared with the spectrum of known components in the library (Fig 3 & Table 3).

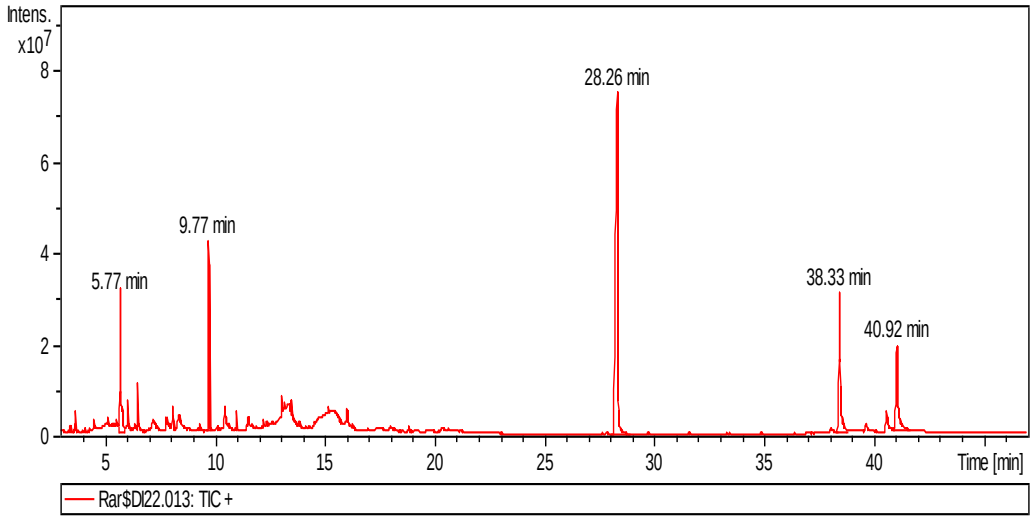

Fig : 3 GC-MS chromatogram of methanolic extract of *Citrus reticulata* peel

Table : 3 Represent the RT, Area & Area percentage of the chromatogram

S.NO	RT(min)	Area	Area %
1	5.77	99044537	18.1
2	9.77	1.08E+08	19.7
3	28.26	5.47E+08	100
4	38.33	1.6E+08	29.2
5	40.92	1.35E+08	24.7

Table 4 List of compounds identified on GC-MS

S.No	RT(min)	COMPOUNDS	Area %
1	3.714	2-furanmethanol	0.66
2	5.771	D-limonene	7.06
3	6.102	Crithmene	1.44
4	6.551	Linalool	0.98
5	8.134	Coumara	1.41
6	9.777	2-methoxy-4-vinylphenol	8.82
7	10.510	Ethanone 1-(2- hydroxyl-5-methylphenyl)	1.66

8	11.007	Tetradecane	0.66
9	11.007	8-methylheptadecane	0.66
10	13.087	4-methyl-2,5-dimethoxybenzaldehyde	2.50
11	13.193	2,3,5,6-tetrafluoroanisole	1.71
12	13.193	Methyl p-tolyloxyacetate	1.71
13	13.501	Hexadecane	2.37
14	15.191	Polygalitol	0.31
15	15.191	Beta-D-glucopyranose	0.31
16	15.191	4-o-beta. D-galactopyranosyl	0.31
17	15.191	N-methoxy-N-acetyl-2-carbomethoxyethylamine	0.31
18	15.191	Pregnenolane	0.31
19	28.263	Bis(2-ethylhexyl)phthalate	46.26
20	38.333	Diisooctyl phthalate	12.11
21	38.333	Beta-sitosterol	12.11
22	40.449	Gamma-sitosterol	2.26
23	40.922	Diisopropylsilyl ether	9.80

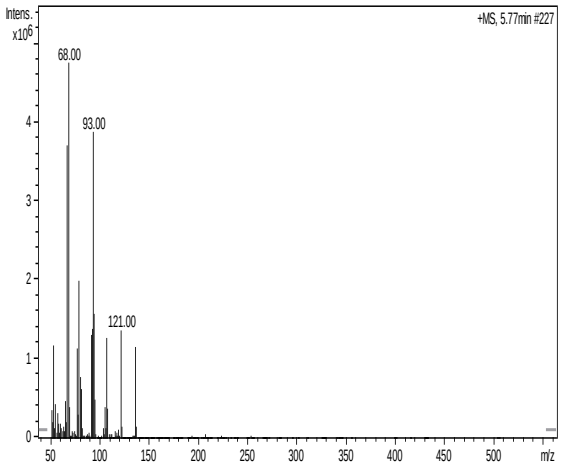

Fig 4: RT(5.77)

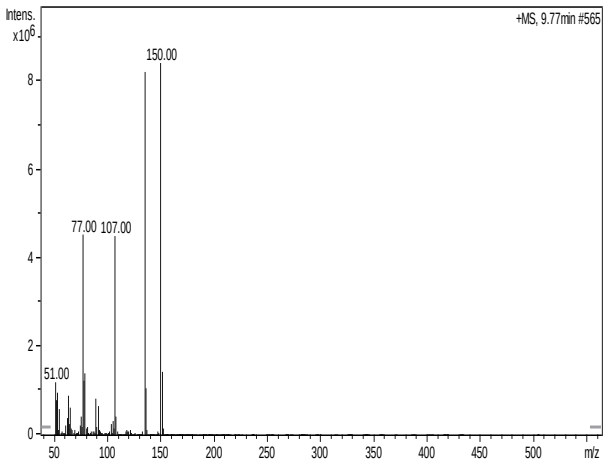

Fig 5: RT(9.77)

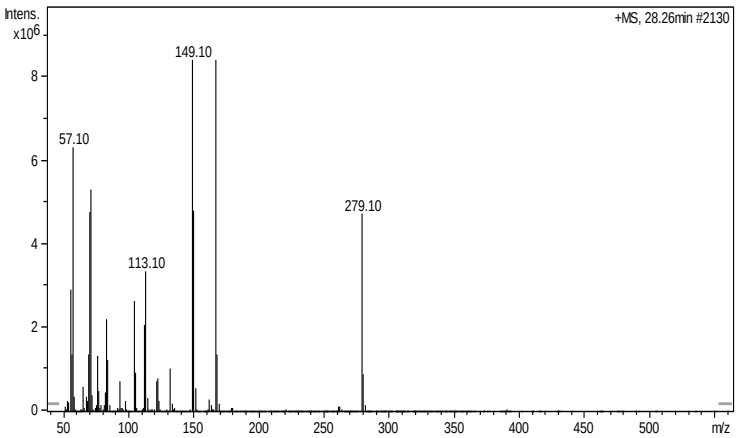

Fig 6: RT(28.26)

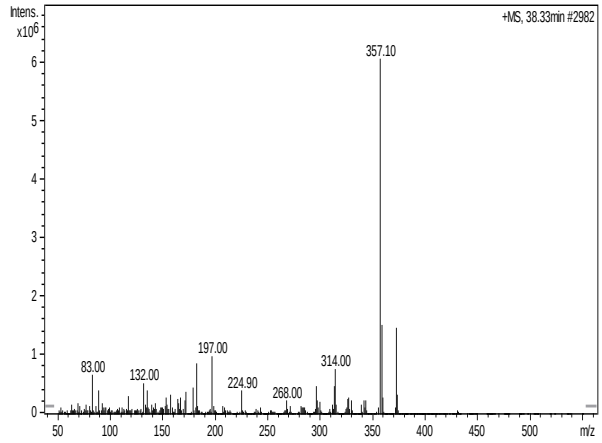

Fig 7: RT(38.33)

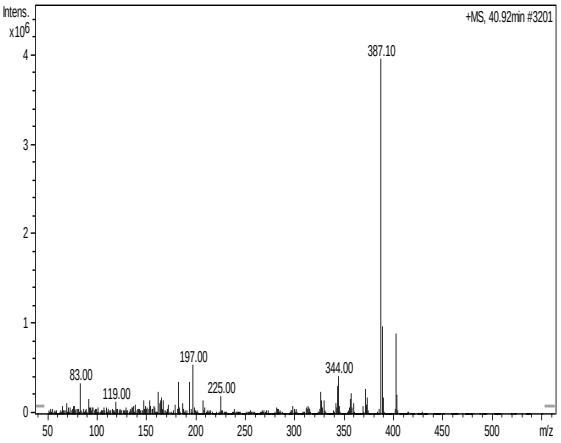

Fig 8: RT(40.92)

Based on GC-MS chromatogram 23 major compounds has been identified and listed Table 4.

CONCLUSION

Spectroscopic technique has become a powerful and analytical tool for the qualitative and quantitative analysis of pharmaceutical and biological materials. *Citrus reticulata* peel is rich in its bioactive compounds than the fruit. It has antioxidant, anti cancer activity.,etc, hence it is very important to know the active compounds present in the peel. UV-VIS spectroscopy, FTIR analysis and GCMS analysis help us to identify the compounds present in peel. UV-VIS is routinely used in analytical chemistry for quantitative determination. FTIR is a strong tool in identifying the chemical structure of the compounds present in the peel. GCMS is an analytical method to identify different substances within a test sample. This attempt is a step to justifying its abundance and could open the door of exploring new clinically strong biotherapeutic agents. Waste is always not a waste, *Citrus reticulata* peel is considered as waste, but the present finding reveals that fruit peel can be an alternative use in pharmaceuticals and food industries.

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