International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Phytochemical Profiling and Molecular Authentication of *Elaeocarpus variabilis Zmartzy* from Western Ghats (Ooty)

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Abstract: ***The Western Ghats, renowned for its rich biodiversity, harbors numerous plant species with both aesthetic beauty and significant medicinal properties. Many of these plants have demonstrated promising results in treating various diseases with minimal or no side effects. However, several underutilized plant species in the Western Ghats remain largely consumed and utilized only by indigenous communities. These plants have been traditionally employed as remedies for a wide range of ailments and, in some cases commercialized as medications for various diseases and disorders. This is popularly referred to as AYUSH, encompassing Ayurveda, Yoga, Unani, Siddha, and Homeopathy, highlighting the efficacy and safety of plant-based medicines. One such underutilized plant, Elaeocarpus variabilis Zmartzy, was selected for study, aiming to uncover its significance. This plant, known as the South Indian Marble tree, is a member of the Elaeocarpus genus, with around 350 species found primarily in India, Southeast Asia, Southern China, and Japan. In India, approximately 25 species exhibit a disjunct distribution, with the Southern group being endemic to the Western Ghats. Elaeocarpus species have diverse traditional uses, including making pickles, chutneys, and Rudraksha beads. They have been used in folk medicine to treat various health issues, such as poison antidotes, antiseptics, digestive disorders, skin ailments, and more. Additionally, Elaeocarpus fruits have been used in Ayurveda for treating bronchitis, migraines, and other conditions. This study focuses on Elaeocarpus variabilis Zmartzy, aiming to profile its phytochemical composition and establish DNA barcodes, contributing to a deeper understanding of this underutilized plants potential benefits.***

Keywords: Western Ghats, underutilized plants, *Elaeocarpus variabilis Zmartzy*, phytochemical analysis, DNA barcoding, traditional medicine

1. Nilgiris A Great Biosphere Reserve

Western Ghats is one of the important hotspots that is both rich in flora & fauna. They not only harbour plants of aesthetic beauty but also of great medicinal use. Plants have been giving promising results in curing many diseases. Another great merit of consumption of plants for medication has no or less side effects. There are a number of underutilised plants in Western Ghats that are been only consumed and utilised by the indigenous people there. These plants have been used by those indigenous people as a curative for a number of diseases. Some of the plants are also been commercialised as medications for many diseases and disorders. It's been famously called the "AYUSH" wherein the A stands for Ayurvedic, Y stands for Yoga, U for Unani ,S for Siddha and H for Homeopathy. As mentioned earlier these plants when used as drugs has less or no side effects and are more reliable than the other drugs. Thus the underutilised plant was taken for study for revealing the importance of it. Few underutilised plants in Western Ghats are namely *Rubus ellipticus Sm., Rhodomyrtus tomentosa, Berberis tinctoria, Elaeagnus kologa, Syzygiumarnottianum* and many others. The underutilised plants are not only wild in nature but also possess high level of nutritional content in it naturally. One of such underutilised plant was taken from Ooty of Western Ghats namely the Queen of Hills. They were then studied based on biochemical & molecular techniques.

The plant that was taken for study was *Elaeocarpus variabilis Zmartzy*.

The word Elaeocarpus come from the Greek word meaning Olive fruited since it resembles the olives. The genus *Elaeocarpus* is one among the evergreen trees and approximately about 350 species are there, that are widespread in areas like India, Southeast Asia, Southern China & Japan. In India there are about 25 species showing disjunct distribution with Northern and Southern group of species. The Southern group of species are endemic to Western Ghats. In many regions the *Elaeocarpus* fruits are been used for making pickles & chutneys. Another important species is the *E.ganitrus* are used for making Rudraksha, a sacred bead. Traditionally different species of *Elaeocarpus sp.*, were used in folk medicine as antidote for poison, antiseptic, diarrhoea, dysentery, epilepsy, gonnorhea, hemorrhages, leprosy, piles, pneumonia, rheumatism, scabies, toothache, typhoid, ulcers as mouth wash, appetizer and so on. Other than the leaves the various parts of the Rudraksha had been used for alleviation of different health problems like mental disorder, headache, fever, skin problems and for healing wounds. The fruits are been used in Ayurveda for treating bronchitis, neuralgia, cephalalgia, anorexia, migraine, manic and are classified as thermogenic, sedative, cough alleviator.

Figure 1

Description about the plant:
Kingdom: Plantae
Class: Tracheophyta
Order: Magnoliopsida
Sub-order: Oxalidales
Family: Elaeocarpaceae
Genus: Elaeocarpus
Species: *Elaeocarpus variabilis Zmartzy*

- *Elaeocarpus variabilis Zmartzy* is also known as the South Indian Marble tree.
- This tree belongs to the Rudraksh family and as the name indicates it has a hardcore seed that resembles the Rudraksh.
- Global distribution: Western Ghats
- They usually grow on the slopes.

- This tree reaches up to 20m & the bark is brownish, warty, wood white to cream(Fig 1) and the leaves are simple, alternate, spiral, clustered at twig ends.
- Some of the leaves turn red as it reaches senescence.(Fig C)
- Flower: Flowers are borne in axillary and terminal branched inflorescence.
- The sepals are 5 that are glandular on the inner side and petals 5 that are dissected into many hair like structures towards the free end.
- Fruit: The fruit generally gives a dark greenish to brownish hue when its fully ripen and green when unripen (Fig B)and is slightly sweet in taste.
- Flowering & fruiting: May-October .
- Usually every village in Nilgiris has 1-2 *Elaeocarpus variabilis* in it and approximately altogether around 820 are there in count.

Figure A

Figure B

Figure C

Figure D

- The Fig A shows the leaves of the species and the Fig D shows the tree species along with the leaves in their habitat.
- This tree serves as an asylum for many endangered, endemic species.
- The long trees not only act as a great rejuvenator of air but also plays a major role in protecting the soil.
- The long roots helps the soil from erosion and increases the ground water level.
- It also acts as an elixir since the tree acts a great buffer in the environment and the fruit when consumed acts as a booster.
- Traditionally the fruits of E.variabilis Zmartzy are been consumed without intense knowledge about the fruit but later they had been using the plant in many ayurvedic systems.

Aim & Objective:

Aim:

The present investigation was aimed to profile the phytochemicals and to identify the DNA barcodes of *E.variabilis Zmartzy.*

Objective:

- Characterisation of phytochemicals present in the fruit pulp of *E.variabilis* Zmartzy using FTIR & GCMS analysis.

- Optimization of DNA isolation ad PCR conditions for DNA barcoding using universal primers (matK, rbcl & ITS)

2. Materials and Methods

The details about the different procedures and techniques followed during the course are listed below.

Collection of sample:

- The sample of both leaves and fruits were collected from Western Ghats(Ooty) specifically from the village Jagathala. (Fig 2)
- The freshly collected samples were immediately packed properly and brought in ice box.
- The tender leaves were used for DNA profiling & the fruits were used for phytochemical analysis.

Figure 2: Fruits and leaves sample of *E.variabilis Zmartzy* collected.

Authentication:

The sample collected was authenticated as *Elaeocarpusvariabilis Zmartzy* by the Botanical Survey of India(BSI),Coimbatore.

DNA Isolation

The DNA isolation was performed with the leaves of the sample using CTAB method. One of the great challenging task was the isolation since the leaves of the sample are very leathery.

DNA isolation was null in 2%CTAB & also in the kit extraction method. Later changes was made & 4%CTAB was used and the result was obtained thus the standardization of DNA isolation was made with the 4% CTAB for *Elaeocarpus variabilis Zmartzy*. Firstly 1g of the sample was weighed and taken for maxi preparation. Liquid nitrogen was used for one set of sample and for another the sample was kept inside cryofreeze for complete grinding. Later 10ml of 4% CTAB was added to it for intense grinding. The tubes were then kept at water bath for 45min at 65°C and intermittent inversion of the tubes were done. Later equal volume of chloroform and isoamyl alcohol in the ratio 24:1 was added and mixed completely by inversion. Later centrifugation @10,000rpm for 15min was done. The supernatant was then collected and equal volume of isopropanol was added and incubated for 1hr @ -20°C or overnight incubation at 4°C is done. Again centrifugation at 10,000rpm for 15min was done and now the pellet was taken. To this 100-200µl of ethanol was added and it was completely dried and later dispensed in water. In case of longtime storage TE buffer could be used. Finally the sample was run in 0.8% agarose in AGE apparatus.

PCR (Polymerase Chain Reaction)

a) PCR amplification is useful in DNA profiling of the novel species.
b) The programming is as follows:
   - Initial denaturation: 94°C for 10 minutes
   - Final denaturation :94°C for 45 seconds
   - Annealing: Gradient temperature from 51-55°C for 30 seconds.
   - Extension:72°C for 30 seconds.
   - Final extension:72°C for 10 minutes
c) The different inputs for the PCR is as follows:
   - PCR master mix: Taq buffer
     dNTP
     Taq polymerase
     Primers: Forward & Reverse
     Template
     Water
d) Four different primers were synthesized with the help of the oligonucleotide sequence from 5' to 3' end and were used:

ITS-F5	AATGGTCCGGTGAAGTGTTC
F5ITS-R2	CTCGCCGTTACTAGGGGAAT
ITS2ITS-F3	CCGTGAACCATCGAGTCTTT
F3ITS-R2	CTCGCCGTTACTAGGGGAAT
matK472F	CCCRTYCATCTGGAAATCTCTTGGTTC
matK1248R	GCTRTRATAATGAGAAAGATTTCTGC
rbcLa-F	ATGTCACCACAAACAGAGACTAAAGC
rbcLa-R	GTAAAATCAAGTCCACCRCG

- The primers were reconstituted by diluting the primers wherein 10µl of the primer is dissolved in 90 µl of water.

- The annealing temperature of the reaction was optimized in accordance to the temperature of the primers.
- At different concentrations and gradient temperatures the PCR was set to reaction.

- PCR master mix: 10 µl
- Primer F : 0.25 µl
- R : 0.25 µl
- DNA : 0.5 µl
- H2O : 4 µl

Fruit Analysis

- The fruit sample was first collected from Western Ghats region & later the pulp was scraped out cleanly with sterile scalpel.
- Later it was placed inside the hot air oven for complete drying and for its crispiness.
- Later with the help of a pulverizer it was completely powdered.
- 40g of the powdered sample was then weighed and then with the help of Soxhlet apparatus the extraction was done.
- Methanol has been used as a solvent for the extraction process.
- The powdered sample was completely packed in a filter paper and inside the hollow tube of the apparatus and the solvent methanol was taken in the round bottomed flask. The former was connected to a cold water source and the latter was placed on a heating mantle.
- As the solvent boils @ 40°C the cold water acts as a condenser and the extract of the solvent moves through the Chiffon tube and then down the round bottomed flask and is retained there.
- Usually three cycles are been followed for the better extract of the fruit sample.
- It takes about one day for the completion of the extraction process.

Figure A

Figure B

Figure C

Figure D

Fig A – Pulp extracted of *E.variabilis Zmartzy*
Fig B - Pulverized powder of the fruit
Fig C - Powder been placed for extraction
Fig D - Boiling & condensation takes place wherein the extract is collected at the bottom

- After the extraction the liquid extract was kept in hot air oven for solvent evaporation @ 50°C
- Later the solid part of the extracted sample was given for FTIR analysis and GCMS at SITRA a Research Institution in Coimbatore for the phytochemical study and the results were obtained.
- The phytochemical results of the sample were then been obtained through tabulations & graphical representations.

3. Result and Discussion

DNA Barcoding:
Genomic DNA isolated from leaves of *E.variabilis Zmartzy*

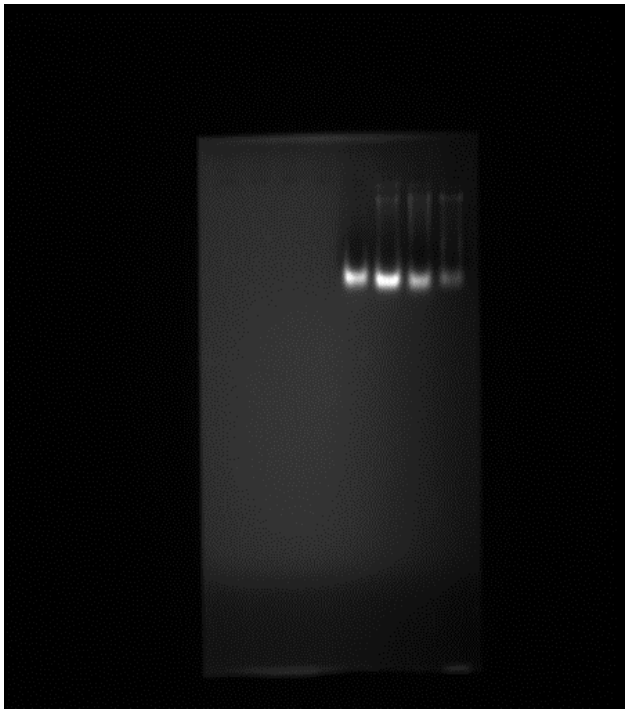


Figure 3: DNA isolated from leaves of *E.variabilisZmartzy*

To identify the DNA barcodes of *E.variabilis Zmartzy*, various universal primer sets (mat K, rbcl and ITS)were used. Optimization of reaction volume and PCR conditions are need to refine for better amplification. Since it is wild tree species isolation of genomic DNA and PCR amplification was very tedious.

A) FTIR Analysis

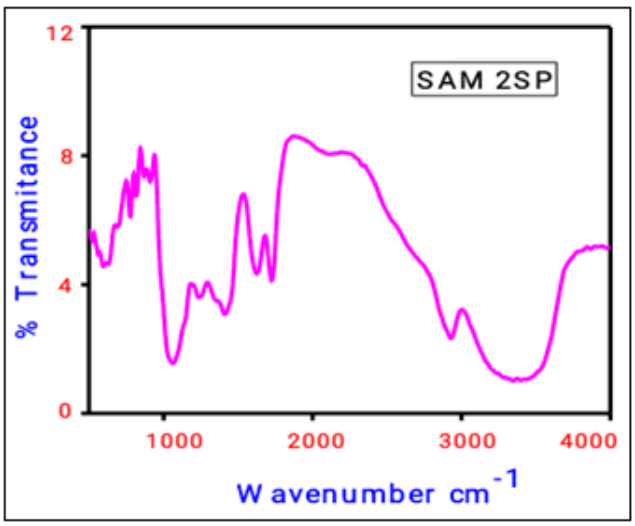


Table 1

Wavelength	Corresponding Compounds
538	Alkali metal ions
858	=C-H out of plane bending
1117	C-C-O stretching
1581	C=O stretching(carboxylate group)
1741	C=O Aromaticring+OH over tone

Table 2

Wavelength range	Compounds
500-730	Halogen compounds(chloro C-Cl)
858	Aromatic compounds
1117	Polysaccharide
1581	Diketones

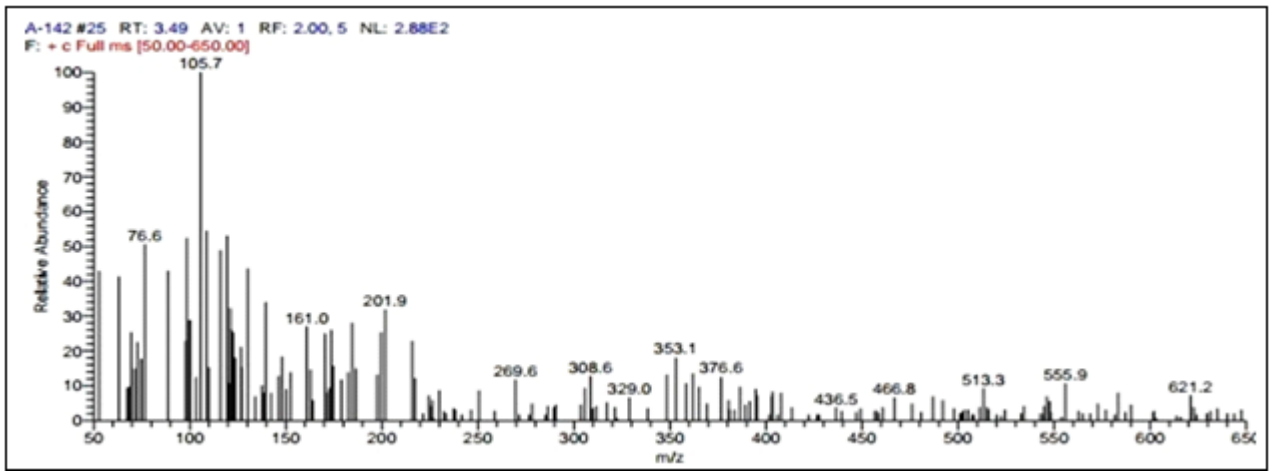
4. Result

The functional groups of the fruit *E.variabilis Zmartzy* were obtained and the results were tabulated according to the standard graph and the standard value for the compounds were tabulated in the Table1 whereas the corresponding compounds are listed in Table 2.

5. Discussion

The major functional groups thus analysed through the FTIR analysis were aromatic compounds, halogen compounds, polysaccharides and diketones. These functional groups are of great importance and has a lot of medical applications in the field of Ayurveda.

B) GCMS:



S.NO.	RT	COMPOUND	M.W	M.F	BIOACTIVITY
1.	3.49	METAL PORPHIN	606	C36H46N5Ni	Main component of heme that helps in O2 supply.
2.	3.49	BENZODIAZEPINE	546	C32H27BrN4	It enhances the role of neurotransmitters & helps in treating sleep disorders.
3.	3.49	PYRROLE	544	C36H26C12O	Intermediate in synthesis of pharmaceutical, medicines & dyes.
4.	3.49	HOMOESTRODIOL DERIVATIVES	606	C32H51FO2Sn	Helpful in women during pregnancy and prevents osteoporosis
5.	3.49	FRAESEOL	206	C12H14O3	Used in fragrance industry, artificial flavors and also incosmetics.
6.	5.92	THIOPENE-OL DERIVATIVES	290	C12H19BrOS	Used in manufacturing dyes aroma compounds & pharmaceuticals.
7.	5.92	CANNABINOIDS	675	C28H25D3F12 O5	They are used as relief for pain, nausea and anxiety.
8.	6.59	BENZOXAZINE DERIVATIVE	191	C11H13NO2	Used as anti-inflammatory and antimicrobial agent.
9.	6.59	BICYCLOAMINE DERIVATIVE	625	C33H47NO3Sn	Useful as therapeutic agents and analgesics for various kinds of neuralgia, neuropathy, epilepsy, insomnia
10.	8.52	STENOPHYLLINE	673	C37H55NO10	Used to cure asthma

6. Result

The fruit sample was analysed through GCMS and a number of components were obtained and the compounds showing major peaks were tabulated.

7. Discussion

The major components obtained were metal porphin, pyrrole, fraeseol, benzodiazepine and the bioactivity was listed. This data helps in suggesting the consumption of the fruit by applying its activity.

8. Summary and Conclusion

The FTIR and GCMS analysis were performed for the fruit sample and the results revealed the phytochemicals present in the sample. The data obtained through the analysis paves way to the better and viable use of the underutilized fruit *E.variabilisZmartzy*.

In future, by the retrieved phytochemical data and the bioactivity the fruit could be used more widely used in treatment and commercialized for the betterment of human race by improvising the health status of the society.

Appendices:

a) CTAB BUFFER (4%) in 100ml H2O
- CTAB - 2g
- 1%PVP- 1g
- 100mM Tris HCl-1.5g (pH 8.0)
- 1.4M NaCl-8.18g (pH 8.0)
- 20mM EDTA-0.7g
- 0.1%β Mercaptoethanol-0.1g

b) TAE BUFFER(50X)
- Tris - 242g
- EDTA – 57.1ml (pH 8.0)
- Glacial – 100ml of 500mM Acetic acid

c) TE BUFFER
- Tris HCL (10mM) -0.15g
- EDTA (1mM) -0.0372

Abbreviations:
- % - percentage
- ml - millilitre
- µl - microlitre
- M - Molar
- mM – millimolar
- min – minutes
- °C - Celsius
- : - ratio
- g - gram
- rpm – rotations per minutes
- Taq -*Thermus acquaticus*
- dNTP -deoxynucleotide triphosphate.

Acknowledgement
First and foremost, I would like to thank my family members for their love, care, constant support and encouragement through all my endeavors, without their support it is impossible for me to finish my college and graduate education seamlessly.

Next I would like to thank the Almighty for having made possible the completion of my project work.

With immense respect, indebtedness and gratitude, I place my sincere and heartfelt thanks to my guide Dr.G.Kapildev, Assistant Professor, Department of Microbial Biotechnology, School of Biotechnology and Genetic Engineering , Bharathiar University, Coimbatore, for his inspiring guidance, for providing all the necessary facilities to carry out the work.

I also extend my sincere love and heartful thanks to all my friends for their support and love during the course of study. It is my pleasant responsibility to thank all my neighbours and relatives who had helped me during the sample collection for my project.

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