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DETERMINATION OF BIOACTIVE COMPOUNDS IN LEAF EXTRACT OF *SYZYGIUM TRAVANCORICUM* GAMBLE- AN ENDANGERED TREE SPECIES USING GAS CHROMATOGRAPHY AND MASS SPECTROSCOPIC TECHNIQUE

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ABSTRACT

Syzyium travancoricum, belongs to the family Myrtaceae, is a critically endangered tree species found in marshy swamps of Western Ghats. *Syzygium travancoricum* has long been revered as medicinal plant among the local population, owing to its valuable medicinal properties. However, the phytochemical constituents and biological activity present in the plant extract of *Syzygium travancoricum* have not been extensively studied. The aim of this study is to identify phytochemical components present in the ethanolic extract of the leaves from *Syzygium travancoricum* using Gas Chromatography and Mass Spectroscopy (GC-MS) technique. GC MS analysis of the ethanolic extract of the leaves from *Syzygium travancoricum* was performed using Nexis GC – 2030 and a Gas Chromatograph interfaced to a Mass Spectrometer. The purpose of this study was to identify the potential chemical constituents present in the leaf extract of *Syzygium travancoricum*. This analysis revealed that the plant extract contains many phytocomponents. It mainly consists of Diethyl azodicarboxylate (26.3%) whose byproducts are considered as a potent antiviral and antitumor agent. The plant extract also consists of other phytocomponents such as 1,3,6-Octatriene,3,7-dimethyl-(Z), Caryophyllene, Naphthalene,Decahydro-4a-methyl-1-methylene-7-(1-methylethyl)-, Bicyclo[7.2.0]undec-4-ene,4,11,11- trimethyl-8-methylene-, Caryophyllene oxide, Phytol, 1OE,12Z-Octadecadienoic acid, 1H-Benzoimidazole,5-ethoxy-2-phenethylsulfanyl-, 1H-Pyrazole,4,5-dihydro-1,3,5-triphenyl-, Myrtenyl caprate, Squalene. The mass spectra of all the phytochemicals present in the plant extract was matched with the National Institute of Standards and Technology (NIST) library. From this analysis, it is proved that *Syzygium travancoricum* plant extract contain many bioactive compounds. If this plant is protected from its extinction threats, it can be used in many pharmaceutical, cosmetic and fragrance industries.

INTRODUCTION

Syzygium travancoricum Gamble is an endangered plant species endemic to the Southern Western Ghats of the Indian subcontinent [1]. This medium sized tree is found at an elevation of 1000-1500 meters in the Shoalas of a tropical evergreen forest. *Syzygium travancoricum* has a well-established medicinal reputation. Locals have traditionally employed this plant species to treat arthritis and diabetes. The astringent, hypoglycemic, antibacterial, antifungal and neuropsychopharmacological properties of this species are well documented [2]. The plant faces significant threats because of its poor germplasm, high outbreeding rate and environmental specialization, which results in low seed viability and regeneration. The primary cause of the significant drop in *Syzygium travancoricum* population are habitat destruction and overexploitation [3]. A significant area of virgin forest is being converted to agriculture, and pressure from grazing, forest fires, and exotic plants has also resulted in a drop in regeneration [4]. Since it is difficult for this tree species to proliferate in large quantities through normal breeding and other means, clonal propagation may be a practical substitute [5].

Phytochemicals are organic compounds derived from plants, can be classified into primary and secondary constituents based on their involvement in plant metabolism based on their involvement in plant metabolism. Primary constituents consist of common sugars, amino acids, proteins, chlorophyll, etc., Secondary constituents encompasses alkaloids, terpenes and phenolics. Research continually uncovers more remarkable benefits of phytochemicals, hinting towards their potential nutritional significance and disease resisting capacities. Over the past decade, significant advancements in analytical techniques, including HPTLC, FT-IR, HPLC, GC-MS, NMR, have emerged as powerful tools for separating, identifying and determining the structure of phytochemicals [6].

With more advanced integrated, automated sample preparation and extraction techniques, GC-MS instrumentation is the unquestionable workhorses in analytical laboratories, made major advancements and enhanced matrix robustness, selectivity for

actual samples and the expanding possibility of multi-compound approaches. The exceptional efficacy of GC is attributed to its ability to separate mixtures of substances and generate transient signals for data deconvolution. The utilization of fused silica columns has elevated GC to the forefront as the paramount and potent method for analyzing intricate mixtures of products. GC-MS perfectly caters to the prevailing inclination towards multi-methods or mutli-component analyses [7]. The aim of this study is to determine the phytoconstituents present in *Syzygium travancoricum* leaf extract with the aid of GC-MS technique.

MATERIAL AND METHODS

COLLECTION OF PLANT SAMPLE

Syzygium travancoricum Gamble used for this study was collected from Jawaharlal Nehru Tropical Botanical Garden & Research Institute (JNTBGRI), Thiruvananthapuram, Kerala. The plant was authenticated by Dr.E.S.Santhosh kumar, Systematic Botanist, Jawaharlal Nehru Tropical Botanical Garden & Research Institute (JNTBGRI), Thiruvananthapuram, Kerala.

PREPARATION OF PLANT EXTRACT

The newly harvested leaves underwent a thorough cleasing process using running tap water. Subsequently, they were carefully dried in an oven at a controlled temperature of 40° C. Once completely dried, the leaves were coarsely ground using a grinder. The resulting powder was then subjected to extraction using absolute ethanol in a soxhlet extractor, maintaining a temperature range of 40-50° C. The extracted solution was subsequently dried on a water bath at 60° C. To further analyze the composition of this dried ethanol extract, GC-MS analysis was conducted.

GC-MS ANALYSIS

The extract was dissolved in mixture of solvents and then subjected to GC-MS analysis. The mixture of solvents involved toluene, ethanol, chloroform and ethyl acetate. The GC-MS of sample was carried out in School of Chemistry (SOC) Instruments Maintenance, Department of Chemistry, Madurai Kamaraj University, Madurai, Tamil Nadu. Sample analysis was done using Nexis GC – 2030 Gas Chromatograph interfaced to a Mass Spectrometer. Inert gas Helium (99.99%) was used as carrier gas. The sample size of 1µl was injected through injector. The total run time of GC was 28 minutes. Data handing was accomplished utilizing the GC-MS software. The determination of compounds was established by comprehensive libraries of National Institute of Standards and Technology (NIST).

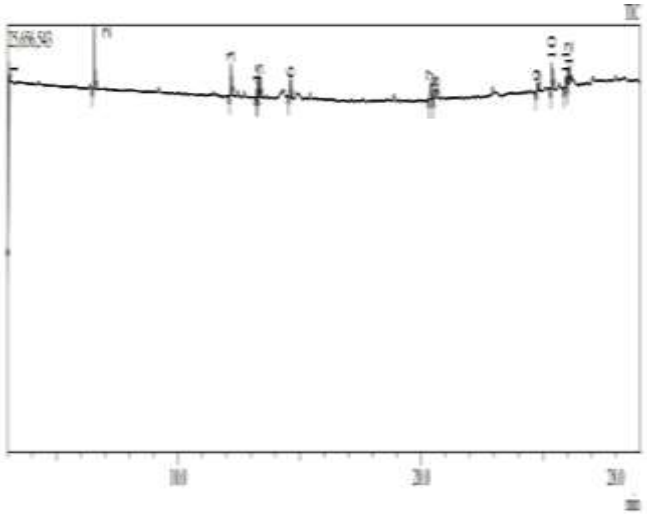

Figure 1: GC-MS chromatogram of leaf extract of *Syzygium travancoricum*

Results and Discussion

Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful analytical technique that merges the capacities of gas-liquid chromatography and mass spectrometry to discern various compounds present in a given sample [8]. Over the recent years, GC-MS has gained significant recognition as a fundamental technological tool for characterizing secondary metabolities in both plant and non-plant organisms [9]. The ethanolic extract of *Syzygium travancoricum* was analyzed using GC-MS chromatography, showing a total of 12 peaks (**Figure 1**). These peaks were then identified by comparing their mass spectra with databases of NIST libraries (**Table 1**).

Table 1: Identification of phyto-constituents present in ethanolic leaf extract of *Syzygium travancoricum*

Peak #	R. Time	Area %	Height %	Molecular weight (g/mol)	Molecular formula	Name of the compound
1	3.046	26.30	25.97	174	C6H10N2O4	Diethyl azodicarboxylate
2	6.556	21.38	20.47	136	C10H16	1,3,6- Octatriene, 3,7-dimethyl-, (Z)
3	12.176	9.75	10.26	204	C15H24	Caryophyllene
4	13.253	2.67	3.14	204	C15H24	Naphthalene, Decahydro-4a-methyl-1-methylene-7-(1-methylethyl)-
5	13.356	5.62	6.53	204	C15H24	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-
6	14.606	5.29	5.74	220	C15H24O	Caryophyllene oxide
7	20.382	5.02	5.08	296	C20H40O	Phytol
8	20.566	4.99	3.02	280	C18H32O2	10E, 12Z-Octadecadienoic acid
9	24.775	2.77	2.69	298	C17H18N2OS	1H-Benzoimidazole, 5-ethoxy-2-phenethylsulfanyl-
10	25.376	7.26	8.46	298	C21H18N2	1H-Pyrazole, 4,5-dihydro-1,3,5-triphenyl-
11	25.957	4.26	3.40	306	C20H34O2	Myrtenyl caprate
12	26.079	4.70	5.24	410	C30H50	Squalene

From the results, it was observed that Diethyl azodicarboxylate and 1,3,6- Octatriene, 3,7-dimethyl-, (Z) were the major components in the leaf extract of *Syzygium travancoricum*. The other phyto-constituents present in the leaf extract are Caryophyllene, Naphthalene, Decahydro-4a-methyl-1-methylene-7-(1-methylethyl)-, Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, Caryophyllene oxide, Phytol, 10E, 12Z-Octadecadienoic acid, 1H-Benzoimidazole, 5-ethoxy-2-phenethylsulfanyl-, 1H-Pyrazole, 4,5-dihydro-1,3,5-triphenyl-, Myrtenyl caprate, Squalene. The biological activities of selected compounds were listed below **(Table 2).**

Table 2: Biological activity of phyto-constituents present in the ethanolic leaf extract of *Syzygium travancoricum*			
R. time	**Name of the compound**	**Biological activity**	**Reference**
6.556	1,3,6-Octatriene, 3,7-dimethyl-, (Z)	Antifungal, Aromatic	[10]
12.176	Caryophyllene	Analgesic, Antiasthmatic, Anticariogenic, Antiedemic, Antifeedant, Antitumor, Antionychyotic	[11]
13.253	Naphthalene, Decahydro-4a-methyl-1-methylene-7-(1-methylethyl)-	Antimicrobial, Antioxidant and medicine of skin disease	[12]
13.356	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-	Anti-inflammatory, Antimicrobial, Anticarcinogenic, Analgesic, Antioxidant activities	[13]
14.606	Caryophyllene oxide	Antiedemic, Antifeedant, Antiinflammatory, Antitumor, Fungicide, Insecticide, Pesticide	[11]
20.382	Phytol	Antimicrobial, Anticancer, Cancer preventive, Diuretic, Anti-inflammatory	[11]
20.566	10E, 12Z-Octadecadienoic acid	Anticancer, Cancer preventive, Antitumor	[14]
25.376	1H-Pyrazole, 4,5-dihydro-1,3,5-triphenyl-	Antifungal, Antibacterial, Anti-tuberculosis, Anti-inflammatory, Antioxidant, Analgesic, Anticancer	[15]
26.079	Squalene	Anticancer, Antimicrobial, Antioxidant, Chemopreventive, Pesticide, Antitumor	[11]

DEAD, also known as diethyl azodicarboxylate, is an azodicarboxylate that plays a crucial role in the Mitsunobu reaction, which is highly versatile and enables the total synthesis of various natural products, drugs, analogues, and semisystematic derivatives derived from naturally occurring compounds [16]. Moreover, Mitsunobu reaction serves as a pivotal step in the synthesis of numerous natural products and pharmaceuticals [16]. The pleiotropic properties of Squalene present in the leaf extract can be further enhanced through its combination with other bioactive compounds and this synergistic effect holds great potential for the development of functional foods and nutraceuticals aimed at controlling oxidative stress and combating age-released diseases in both human and veterinary medicine [17].

Caryophyllene oxide (CO), found in numerous essential oils derived from plants, has received FDA approval as a preservative for both food and cosmetics [18]. Additionally, the European Council has included it in the list of natural and synthetic flavouring substances [19]. Caryophyllene oxide demonstrates anti-inflammatory, anti-carcinogenic and skin-improving characteristics [20].

Phytol and its derivatives demonstrate a diverse array of bioactivities, encompassing anti-anxiety, cytotoxic, metabolism modulating, antioxidant, autophagy and apoptosis-inducing, anti-nociceptive, anti-inflammatory, immune-modulating and antimicrobial effects [21]. In addition to its pharmacological benefits for treating diseases, phytol has been found to possess the potential to combat hyperpigmentation [22].

The unique flavour can be credited to the existence of flavour or aroma compounds present in the plant extract such as 1,3,6-Octatriene, 3,7-dimethyl-, (Z), 10E, 12Z-Octadecadienoic acid, Squalene, Caryophyllene and myrtenyl caprate. It is reported that 10E, 12Z-Octadecadienoic acid, which is isolated from the calyx of eggplants, exhibits cytotoxic activity against human ovarian (HRA) cells (Zhao *et al.*, 2015).

CONCLUSION

Syzygium travancoricum is a medium sized tree found in the marshy swamps of Western Ghats, Kerala. *Syzygium travancoricum* is renowned for its extensive medicinal properties and has earned a well-deserved reputation in the field. It is recognized for its therapeutic properties also. Locally, this plant has a history of being used to alleviate diabetes and arthritis. It is renowned for its astringent, hypoglycemic, bactericidal, antifungal, and neuropsychopharmacological benefits. *S.travancoricum* exhibits antibacterial properties. *Syzygium travancoricum* is reported to be an endemic species that is critically endangered. According to IUCN Redlist 2010, 2012 & 2013 only 200 trees are found in Western Ghats. The plant is confronted with substantial challenges due to its inferior genetic material, high rate of outbreeding, and specialization to specific environments leading to low seed viability and regeneration.

The GC-MS technique employed to analyse the extracts can serve as a valuable tool for quantifying specific active compounds present in plants utilized in various industries such as cosmetics, pharmaceuticals, food, as well as environmental and forensic applications. The GC-MS analysis outcome indicates that the ethanol extract derived from the leaves of *Syzygium travancoricum* contains a diverse range of bioactive compounds. These compounds possess various medicinal properties that hold potential for the treatment of different diseases. From this study, it was concluded that if *Syzygium travancoricum* is protected from its extinction crisis, it can be used in various pharmaceutical and fragrance industries.

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Conflict of interests: None

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Ethical statement: None

REFERENCES

- Roby T.J. Joyce Josh and P. Vijayakumaran Nair (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 od Science, Environment ISSN 2278-3687 (O) and Technology,* Vol. 2. No 6, 2013, 1335-1352.
- Rajalakshmi P, Sumathi V, Vadivel V and Pugalendhi M (2016). Determination of Nutraceuticals in Tropical Medicine Plants of *Syzygium jambos* L., (Alston) and *Syzygium travancoricum* Gamble. *International Journal of Herbal Medicine* 2016; 4(6):150-152.
- Sreekumar V B, Sreejith K A, Sanil M S, Harinarayanan M K, Prejith M P & Varma R V. (2020) Distribution of *Syzygium travancoricum* Gamble (Myrtaceae), a Critically Endangered tree species from Kerala part of Western Ghats, India. *Journal of Threatened Taxa* 2020; Vol. 12; 17340-17346.
- Udhayavani, C., Ramachandran V S., & John H A., (2013). Ecology and population study on *Syzygium travancoricum* Gamble (Myrtaceae)- A Critically Endangered species from Nilgiris. *NeBIO* 4: 68-71.
- Anand, A., Rao, C., & Balakrishna, P., (1999). *In vitro* propagation of *Syzygium travancoricum* Gamble – an endangered tree species. *Plant cell, Tissue and Organ Culture (PCTOC),* 56(1), 59-63. http://doi.org/10.1023/a:1006235900696
- Sweetlin, P., Daniel, R.R., (2020). Determination of bioactive compounds in ethanolic extract of callus derived from *Mucuna pruriens* using gas chromatography and mass spectroscopic technique. *J. Natural remedies* 21(7 (S2), 11-16.
- Hubschmann, H. (2015). Handbook of GC-MS: Fundamentals and Applications. Germany: Wiley.
- Kell, D.B., Brown, M., Davey, H.M., Dunn, W.B., Spasic, I., Oliver, S.G., (2005) Metabolic footprinting and

systems biology: The medium is the message. *Nat Rev Microbiol*.; 3:557-65.

- Fernie, A.R., Trethewey, R.N., Krotzky, A.J., Willmister, L. (2004)., Metabolite profiling: From diagnostics to systems biology. *Nat Rev Mol Cell Biol*. 5:763-9.
- Cavaleiro, C., Salgueiro, L., Goncalves, M.J., *et al*., (2015). Antifungal activity of the essential oil of *Angelica major* against *Candida*, *Crptococcus*, *Aspergillus* and dermatophyte species. *J Nat Med* 69, 241-248. https://doi.org/10.1007/s11418-014-0884-2
- Dr.Dukes. Phytochemical and Ethnobotanical Databases. Phytochemical and Ethnobotanical Database. *www.ars-gov/cgi-bin/duke/* . 2019.
- Manikandan, G., Saranya. M., & Gayathri, S. (2020). GC-MS Analysis of Phyto-Constituents of the Essential Oil from the Leaves of *Melaleuca citrina* (Curtis) Dum.Cours. *Advances in Zoology and Botany,* 8(3), 87-98. https://doi.org/10.13189/azb.2020.080303
- Mahajan, E., Singh, S., Diksha, Kaur, S., & Sohal, S.K. (2022). The genotoxic, cytotoxic and growth regulatory effects of plant secondary metabolite ß-caryophyllene on polyphagous pest *Spodoptera litura* (Fabricius) (Lepidoptera:Noctuidae). *Toxicon*, 219, 106930. https://doi.org/10.1016/j.toxicon.2022.09.016
- Zhao, B., Tomoda, Y., Mizukami, H., *et al.*, (2015)9-Oxo-(10E, 12E)-Octadecadienoic acid, a cytotoxic fatty acid ketodiene isolated from eggplant calyx, induces apoptosis in human ovarian cancer (HRA) cells. *J Nat Med* 69, 296-302. https://doi.org/10.1007/s11418-015-0892-x
- Manojmouli. C., Sai Bharath. V.M., Munazilla Khaleel, T.Y., Pasha, B. Ramesh and K.Naga Prashant. (2022). Recent Updates on Green Synthesis of Pyrazole Derivatives: A Review. *Indian Journal of Natural Sciences*, 13(0976-0997).
- Munawar, S., Zahoor, A. F., Ali, S., Javed, S., Irfan, M., Irfan, A., Kotwica-Mojzych, K., & Mojzych, M. (2022). Mitsunobu Reaction: a powerful tool for the synthesis of natural products: a review. *Molecules,* 27(20), 6953. https://doi.org/10.3390/molecules27206953
- Lou-Bonafonte. J.M., Martinez-Beamonte, R., Sanclemente, T., Surra, J.C., Herrera-Marcos,L.V., Sanchez-Marco, J., Arnal, C., & Osada, J. (2018). Current Insights into the Biological Action of Squalene. *Molecular Nutrition & Food Research*, 62(15). https://doi.org/10.1002/mnfr.201800136
- Food Drug Administration. Washington, DC, USA. Rules and Regulations : title 21 – Food and drugs, food additives, synthetic flavoring substances and adjuvants. Fed Regist (17 May) 1973: 95(38): 12913-12914.
- Yang, D., Michel, L., Chaumont, JP. *et al*. (2000) Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onychomycosis. *Mycopathologia* 148, 79-82. https://doi.org/10.1023/A:1007178924408
- Opdycke DLJ, Letizia C. (1983). Monographs on fragrance raw materials, Caryophyllene oxide. *Food Chem Toxicol*; 21(5): 661-662.
- Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, A. Z. M. T., Ahmed, I. I. K., Shill, M. C., Karmakar, U. K., Yarla, N. S., Khan, I. N., Billah, M. M., Pieczynska, M. D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Banach, M., . . . Atanasov, A. G. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, *121*, 82–94. https://doi.org/10.1016/j.fct.2018.08.032
- Ko, G.A., Cho, S.K., (2018). Phytol suppresses melanogenesis through proteasomal degradation of MITF via the ROS-ERK signalling pathway. *Chem. Biol. Interact*. 286, 132-140.