**PHYTOCHEMICAL PROFILING OF ATHIMATHURA**

**CHOORANAM A POLYHERBAL FORMULATION: A GC-MS ANALYSIS**

 **Abstract**

**Background:** Athimathura Choornam, a traditional Siddha formulation, has been employed for centuries to treat various ailments. **Aim:** This study is aimed to investigate the phytochemical profile of Athimathura Choornam by using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. **Methods:** Gas chromatography-mass spectrometry (GC-MS) analysis was conducted on a Nexis GC 2030 MS AP 2020 NX instrument, by utilizing reagents including HPLC -grade ethanol, concentrated sulfuric acid, concentrated nitric acid, and HPLC-grade water. Sample preparation involved diluting 1g of sample in 50ml ethanol, followed by filtration using helium gas as the carrier." **Results:** The GC-MS chromatogram revealed 10 bioactive compounds, including squalene compounds. The identified compunds are Alpha.Terpinyl acetate,(Terpenoid)1,3 Cyclohexadine,Benzene propanoic acid (belongs to Aromatic carboxylic acids),Butan 2 one(Ketones),2,4- decadienamide(amide group),2-[4-methyl-6(2,6,6-trimethyl cyclohex-1enyl)hexa-1,3,5-trienyl](belongs to Terpenoids,Sesquiterpenes),1-(4-hydroxy-3methoxyphenyl)dec-4-en-3one(Phenylalkanones),Retrofractamide-A(Belongs to alkaloids, amides and Peptides),5-hydroxy-1-(-4-hydroxy-3-methoxy phenyl)decan-3one(Phenylalkanones), squalene(Terpenes group). The compounds were identified by quantifying using peak area and internal standard methods. The results revealed the presence of therapeutically relevant compounds, by substantiating the traditional uses of Athimathura Choornam. **Conclusion:** This study provides a capacious phytochemical profile of Athimathura Choornam, paving the way for further pharmacological and clinical investigations.

**Keywords**: Athimathura Choornam, GC-MS, Phytochemical profiling, Squalene compounds, Terpenoids.

**1.BACKGROUND**

Athimathura Chooranam, a traditional Siddha formulation, has been hired for years to treat various afflictions, including respiratory and gastrointestinal disorders. According to Siddha Literature , Athimathura Chooranam is a traditional herbal remedy that is used to relieve nausea,vomiting,head aches, Pitham and skin irritation. The formulation is allocated to its complex mixture of bioactive compounds, which remain largely unexplored. Gas Chromatography-Mass Spectrometry (GC-MS) is a powerful interpretive technique that is capable of identifying the phytoconstituents present in Athimathura Chooranam.

**2.AIM** **AND OBJECTIVE**: To evaluate the phytochemical profile of Athimathura Chooranam using Gas chromatography-Mass Spectrometry (GC-MS) analysis and relate the identified compounds with its traditional therapeutic claims.

 **2.1 OBJECTIVE**:1.To relate the identified compounds with the formulation’s traditional therapeutic claims.

 2.To Evaluate the potential Pharmacological activities of the identified compounds.

**3.MATERIALS AND METHODS:**

**3.1 PREPARATION OF SAMPLE:** The raw drugs were procured from indigenous raw drug store in Chennai and drugs was purified according to Sikicharatnam deepam and Marunthusei iyalum kalaiyum. The purified drugs were made fine powdered by using a pulverizer.

**3.2 INGREDIENTS IN ATHIMATHURA CHOORANAM :** The ingredients in the formulation includes.,Athimathuram-300mg,Inji-200mg,Thipilli-200mg,Elam -100mg,Seeragam-100mg as mentioned in Siddha literature.

**3.3MATERIALSUSED:** Athimathura Chooranam, Reagents Used : i)Ethanol,ii)Methanol(HPLC Grade Alcohol),iii)Concentrated Nitric acid,iv)Concentrated hydrochloric acid,v)Sulphuric acid,vii)Water(HPLC grade water),vii)GC MS Instrument name : GC –Nexis 2030 MS –QP 2020 NX ,viii)Carrier gas : Helium gas,Column name : SH Rxi-5Sil MS, Column Max temp : 320.0 0,Length : 30.0m,Inner diameter :0.25mmID,Film thickness : 0.25µm Split Ratio :1:10,Flow rate :1:20 ml/min .

 **3.4 METHODOLOGY**:

* Sample Preparation : One gram of sample was diluted with 50 ml of Methanol.(HPLC grade )
* The Athimathura Chooranam sample was analyzed using GC-MS following standard procedures. Biological compounds in the sample were identified using a Nexis GC 2030 Gas Chromatograph with a thermal desorption system (TD20) coupled with a mass spectrometer (Shimadzu). The ionization voltage was set to 70 eV, and the GC was run in temperature programming mode with a Restek column (0.25 mm, 60 m, XTI-5). The initial column temperature was set to 60°C for 2 minutes, then increased linearly to 70°C, followed by a rise to 200°C, which was held for 3 minutes. The temperature was further increased linearly to 280°C, which was held for 10 minutes. The injection port temperature was 290°C, and the GC/MS interface was maintained at 29°C. The sample was introduced using an all-glass injector in split mode, with helium as the carrier gas at a flow rate of 1.20 mL per minute. Identification of metabolites was based on comparing retention times and fragmentation patterns with mass spectra from the NIST spectral library (version 1.10 beta, Shimadzu) in the GC-MS software. The relative percentages of each constituent in the extract were determined by peak area normalization.

**3.5 GC-MS Step:** The filtered liquid is injected into the GC-MS Machine.Gas Chromatography seperates the compounds based on their properties,so that they flow one by one.

**3.6 Analysis with mass spectrometry**: As each compound exists the GC,it goes into the Mass Spectrometer.The Mass Spectrometer breaks down the compound into ionized fragments,creating a unique finger print for each compound.

**3.7 Identification** : The GC-MS Software compares these fingerprints with a library of known compounds to identify them.This process allows to identify which compounds are present in Athimathura Chooranam.

**3.8 DATA ANALYSIS AND RESULTS OF GC-MS**

* After the GC-MS run,collect the chromatogram(the GC output showing compound separation)and mass Spectrometer(MS data with fragment ions).
* The data shows the peak representing each compound,where each peak corelates to a retention time.
* This matching process identifies each compound in Athimathura chooranam and provides their chemical names.
* A number of ten bio active compounds were identified.

Figure 1: Gas Chromatography of Athimathura Chooranam

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Peak | Retention.Time | PeakArea | %PeakArea | Compound name |
| 1. | 11.290 | 899460 | 4.16 | alpha.-Terpinyl acetate |
| 2. | 13.230 | 764044 | 3.53 |  1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R\*,S\*)]-  |
| 3.  | 14.811 | 766238 | 3.54 | Benzenepropanoic acid, 4-hydroxy- |
| 4. | 14.990 | 488811  | 2.26 | Butan-2-one,4-(3-hydroxy-2-methoxyphenyl)- |
| 5. | 18.811  | 5194238  | 24.02 | 2,4-Decadienamide, N-isobutyl-, (E,E)- |
| 6. |  23.657 | 704122 | 3.26 | 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1 |
| 7. | 23.743 | 581099 | 2.69 | 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one |
| 8. | 24.274  | 10104889 | 46.74 |  Retrofractamide-A  |
| 9. | 24.732  | 1414127  | 6.54 | 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one |
| 10. | 28.572 | 704223 | 3.26 | Squalene |

TABLE 1: Retention time and peak area of identified bio active compounds

|  |  |  |  |
| --- | --- | --- | --- |
| COMPOUND NAME | FORMULA | MOLECULAR WEIGHT | DESCRIPTIONS |
| Alpha.Terpinyl Acetate | C12H20O2 | 196.29g/mol | Cardio vascular and Anti-hypertensive effectAnti-cancerAnti-convulsant activityAnti-microbial activity[1] |
| 1,3-Cyclohexadiene | C6H8 | 80.13gm/mol | Neuroprotective activityAnti-InflammatoryActivityAnti-cancer[2] |
| Benzenepropanoic acid, | C9H1003 | 164.20gm/mol | Anti-neuro inflammatory activity [3] |
|  Butan-2-one  | C4H80 | 72.11gm/mol | Polar aprotic solventBacterial Metabolite[4] |
| 2,4-Decadienamide(Pellitorine) |  C14H25N0 | 223.35gm/mol | Antithrombotic activity[5]Anti-skin cancer activity(recent studies) [6] |
| 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl) hexa-1,3,5-trienyl] cyclohex-1 | C23H32O | 324.5gm/mol | Anti-microbial activityPrenyl-diphosphate inhibitorRetinol dehydrogenase inhibitorUbiquinol-cytochrome-c reductase inhibitor[7] |
| 1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one (6-Shogaol) | C17H24O3 | 276.37gm/mol | Inhibits P300 histone acetyltransferase activity [8]Anti-cancer activityAnti microbial activityAnti-inflammatory activity |
| Retrofractamide-A  | C20H25N03 | 327.4gm/mol | Anti-amoebic activity[9] |
| 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (6 Gingerol)  | C17H2604 | 294.4gm/mol | Anti-tumor,anti-inflammatory,Anti-ulcer activity[10] |
| Squalene  | C30H50 |  | Essential for synthesis of steroid hormones,Vitamin D,immunologic adjuvant in several vaccines,including malaria,HIV[11][12] |

TABLE 2: Detail descriptions of bio active compounds

**4.DISCUSSION**

The GC-MS analysis of Athimathura Chooranam revealed a diverse array of bioactive compounds, including terpenoids and fatty acids. Tables 1 and 2 present the identified bioactive compounds along with their medicinal applications. The findings suggest that the compounds in Athimathura Chooranam possess significant biological activities, such as anti-hypertensive, anti-thrombolytic, anti-cancer, antimicrobial and other effects. Specifically, the compound Alpha terpinyl acetate demonstrates cardiovascular and anti-hypertensive properties. Oral administration of Alpha terpinyl acetate has been shown to lower mean arterial pressure and induce vascular endothelium-independent vasodilatation in mesenteric artery rings, along with changes in biochemical parameters indicating antioxidant effects (Sabino et al.).[1]

Additionally, compounds containing 1,3-cyclohexadiene have been associated with a range of bioactivities, including neuroprotective, anti-inflammatory, anti-bacterial, anti-cancer, cytotoxic, and phytotoxic effects (Ignacio et al.). [2]

Neuroinflammation is an inflammatory process occurring in the central nervous system (CNS) and is involved in a variety of complex neurological diseases. Among these, neurodegenerative diseases such as Parkinson's disease (PD), Huntington's disease (HD), and Alzheimer's disease (AD) significantly impair quality of life, particularly for the elderly. There is an urgent need to explore new potential anti-inflammatory treatments, such as those derived from *Citrus grandis*. Identifying anti-neuroinflammatory compounds from *Citrus grandis* and understanding their mechanisms could be of great significance. Our ongoing search for bioactive compounds has led to the isolation of four new compounds, including prenylated benzene propanoic acid.[3]

The Butan 2 one is a compound which acts as a bacterial metabolite.[4]Pellitorine is one of the bioactive compound which is present in Athimathura Chooranam.The Pellitorine exhibits anti-thrombotic and anti-skin cancer activity.[5,6] 2-[4-methyl-6-(2,6,6-trimethylcyclohex-1-enyl)hexa-1,3,5-trienyl]cyclohex-1 exhibits Anti-microbial activity and it has Prenyl-diphosphate inhibitor activity and Retinol dehydrogenase inhibitor activity ,Ubiquinol-cytochrome-c reductase inhibitor[7]

Top of Form

 6-Shogaol, a bioactive compound found in ginger, inhibits P300 histone acetyltransferase activity and reduces the progression of pressure-overload induced heart failure. In a dose-dependent manner, 6-Shogaol prevented TAC-induced systolic dysfunction and cardiac hypertrophy. Additionally, it significantly reduced the TAC-induced increases in histone H3K9 acetylation. These findings suggest that 6-Shogaol may help alleviate heart failure through various mechanisms, including the inhibition of p300-HAT activity.[8] Retrofractamide-A exhibits anti-amoebic activity.[9] 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)decan-3-one (6 Gingerol) has Anti-tumor,anti-inflammatory,Anti-ulcer activity[10]

Squalene, a hydrocarbon primarily derived from shark liver oil and various plant sources, is increasingly being used as an immunologic adjuvant in several vaccines. It has also been widely incorporated into cosmetic formulations as an emollient, antioxidant, and hydrator due to its ability to emulsify easily and spread effectively (Giuseppe Lippi et al.).[11,12]

**5.CONCLUSION**

The results and discussion above clearly highlight the role of Athimathura Chooranam in the treatment of various ailments like Neurologic diseases, Infectious diseases, Psychiatric disorders, Skindiseases, etc.,Further research is recommended to isolate individual compounds and assess their pharmacological efficacy. The findings from these studies would help validate the place of Athimathura Chooranam in traditional medicine and encourage further exploration for its integration into modern therapeutic practices.

**6.References**

1.Khaleel, Christina, Tabanca, Nurhayat and Buchbauer, Gerhard. "α-Terpineol, a natural monoterpene: A review of its biological properties" *Open Chemistry*, vol. 16, no. 1, 2018, pp. 349-361. <https://doi.org/10.1515/chem-2018-0040>

2.Tobal, Ignacio & Bautista, Rocío & Diez, David & Garrido, Narciso & garcia garcia, Pilar. (2021). 1,3-Cyclohexadien-1-Als: Synthesis, Reactivity and Bioactivities. Molecules. 26. 1772. 10.3390/molecules26061772.

3.Li J, Duan M, Yao X, Tian D, Tang J. Prenylated benzenepropanoic acid analogues from the Citrus grandis (L.) Osbeck and their anti-neuroinflammatory activity. Fitoterapia. 2019 Nov 1;139:104410–0.

4.PubChem. Methyl ethyl ketone [Internet]. Nih.gov. PubChem; 2019. Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Methyl-ethyl-ketone>

5.Ku SK, Lee IC, Kim JA, Bae JS. Antithrombotic activities of pellitorine in vitro and in vivo. Fitoterapia. 2013 Dec;91:1–8.

6.Mgbeahuruike EE, Yrjönen T, Vuorela H, Holm Y. Bioactive compounds from medicinal plants: Focus on Piper species. South African Journal of Botany. 2017 Sep;112:54–69.

7.Rajkumar, P. & Sundari, S & Selvaraj, Sankar & Natarajan, A & Suganya, R. & Jayaprakash, R & Kasthuri, K. & Kumaresan, Subramanian. (2022). GC-MS, Phytochemical Analysis and In Silico Approaches of a Medicinal Plant Acalypha indica. The Bangladesh journal of scientific research. 14. 671-684. 10.3329/jsr.v14i2.56648.‌

8.Kawase Y, Yoichi Sunagawa, Shimizu K, Masafumi Funamoto, Toshihide Hamabe-Horiike, Yasufumi Katanasaka, et al. 6-Shogaol, an Active Component of Ginger, Inhibits p300 Histone Acetyltransferase Activity and Attenuates the Development of Pressure-Overload-Induced Heart Failure. Nutrients. 2023 May 8;15(9):2232–2.

9.Kumar S, Kamboj J, Suman, Sharma S. Overview for Various Aspects of the Health Benefits of Piper Longum Linn. Fruit. Journal of Acupuncture and Meridian Studies [Internet]. 2011 Jun;4(2):134–40. Available from: [https://www.sciencedirect.com/science/article/pii/S2005290111600204?via%3Dihub](https://www.sciencedirect.com/science/article/pii/S2005290111600204?via=ihub)

10.Samota MK, Rawat M, Kaur M, Garg D. Gingerol: extraction methods, health implications, bioavailability and signaling pathways. Sustainable Food Technology [Internet]. 2024 [cited 2024 Sep 27];

11.Kim SK, Karadeniz F. Biological importance and applications of squalene and squalane. Adv Food Nutr Res. 2012;65:223-33. doi: 10.1016/B978-0-12-416003-3.00014-7. PMID: 22361190.‌

12.Lippi G, Targher G, Franchini M. Vaccination, squalene and anti-squalene antibodies: Facts or fiction? European Journal of Internal Medicine. 2010 Apr;21(2):70–3.

Bottom of Form