EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 12
Issue: 7
Year: 2025

GC-MS AND GC-FID ANALYSIS OF *SALVIA ROSMARINUS* (ROSEMARY) PHYTOCHEMICALS FOR SLEEP -PROMOTING EFFECTS ON INSOMNIAC WISTAR RATS

[*1]Iwuchukwu Cynthia Ngozichukwu, [2]Adaeze Bob Chile-Agada, [3]Iwuchukwu Patrick Ifekandu, [4]Amadi Rejoice Chinazaekpere

[1]Department of Biochemistry, Imo State University, Owerri, Imo State, Nigeria.
[2]Department of Biochemistry, Imo State University, Oweri, Imo State, Nigeria.
[3]Department of Standards and Quality assurance, Imo State Health Insurance Agency, Owerri, Imo state Nigeria.
[4]Department of Nursing Science, Imo State University, Owerri, Imo state, Nigeria.

***Corresponding Author: Iwuchukwu Cynthia Ngozichukwu**
Department of Biochemistry, Imo State University, Owerri, Imo State, Nigeria.

Article Received on 28/05/2025 **Article Revised on 18/06/2025** **Article Accepted on 08/07/2025**

ABSTRACT
Insomnia, a prevalent sleep disorder affecting millions worldwide, was investigated in this study through the lens of *Salvia rosmarinus* (rosemary) phytochemicals' sleep-promoting effects in Wistar rats with theophylline-induced insomnia. Phytochemical analysis using GC-MS and GC-FID revealed bioactive compounds, including flavonoids (kaempferol, catechin, quercetin) and fatty acids (linoleic acid, 24.34%; oleic acid, 12.48%). Insomnia was induced in Wistar rats via intra-gastric administration of 100 mg/kg body weight of theophylline for 3 consecutive days, *ad libitum*. The *Salvia rosmarinus* extract demonstrated significant sedative and hypnotic effects ($p \leq 0.05$), suggesting potential therapeutic applications in managing insomnia and sleep-related disorders due to its antioxidant and anti-inflammatory properties. Further research is warranted to confirm bioactive compound identities, determine optimal dosages, and elucidate underlying mechanisms.

KEYWORDS: *GC-MS, GC-FID, Salvia rosmarinus, Sleep-promoting effects, Insomnia, Wistar rats.*

INTRODUCTION
Sleep is essential for physical and mental health, playing a critical role in cognitive function, mood regulation, and overall well-being (Vandekerckhove & Wang, 2024). Insomnia characterized by difficulty initiating or maintaining sleep, affects millions worldwide, leading to impaired daytime function, decreased quality of life, and increased risk of chronic diseases (Titova et al., 2022). The prevalence of insomnia is estimated to be around 27% globally, with significant personal distress, daytime functional impairments, and physiological dysfunctions (Bjorøy et al., 2020). Current treatments for insomnia include pharmacological and non-pharmacological approaches, such as cognitive behavioral therapy for insomnia (CBT-I), pharmacotherapy, light therapy, and exercise (Khan & Al-Jahdali, 2023; Cheng et al., 2019). However, these treatments have limitations, and there is a growing interest in exploring alternative therapies, including herbal remedies (Ahmed et al., 2020).

Rosemary (Salvia rosmarinus), a perennial herb native to the Mediterranean region, has been traditionally used to alleviate various ailments, including insomnia, depression, and anxiety (Bano et al., 2019). Rosemary is rich in antioxidants and anti-inflammatory compounds, which may contribute to its potential therapeutic effects (Amaral et al., 2019). This study investigated the sleep-promoting effects of *Salvia rosmarinus* phytochemicals using Gas Chromatography-Mass Spectrometry (GC-MS) and Gas Chromatography-Flame Ionization Detection (GC-FID) analysis in Wistar rats with insomnia. Our results demonstrate the potential sleep-promoting effects of rosemary extracts, which were analyzed using GC-MS and GC-FID, powerful analytical techniques used to identify and quantify phytochemicals in plant extracts (Sparkman et al., 2011; Król et al., 2020; Gupta et al., 2019).

The findings of this study have significant implications for human health, suggesting that *Salvia rosmarinus* extracts possess sleep-enhancing properties, which could lead to the development of novel therapeutic approaches for insomnia and other sleep disorders (Srivastava et al., 2010). Additionally, the identification of bioactive compounds in rosemary could provide insights into the development of natural sleep aids, potentially reducing

the reliance on pharmaceutical sleep medications (Kruk et al., 2018).

MATERIAL AND METHODS

The phytochemical composition of *Salvia rosmarinus* was analyzed using a multi-step process involving the preparation of a crude extract. The leaves of *Salvia rosmarinus* were prepared through drying, grinding, and macerating in hot distilled water (60°C) for 24 hours, followed by treatment with ethanol and potassium hydroxide, and subsequent extraction with hexane. The extracts were then subjected to Gas Chromatography-Flame Ionization Detection (GC-FID) on a BUCK M910 Gas Chromatograph and Gas Chromatography-Mass Spectrometry (GC-MS) on a BUCK M910 Gas chromatography, revealing the presence of bioactive compounds, including fatty acids (e.g., linoleic acid, 24.34%; oleic acid, 12.48%). Insomnia was induced in Wistar rats via intra-gastric administration of 100 mg/kg body weight of theophylline for 3 consecutive days, *ad libitum.* Experimental evaluation demonstrated significant sedative and hypnotic effects ($p \leq 0.05$). Data were analyzed using SPSS version 22, with statistical significance set at $p \leq 0.05$. The broader effects and implications suggest that *Salvia rosmarinus* extracts may have therapeutic effect in managing insomnia and sleep-related disorders, potentially attributed to antioxidant and anti-inflammatory effects. Further research is recommended to confirm the identities, quantities, and potential uses of the bioactive compounds and to determine the optimal dosage for potential therapeutic use.

RESULT

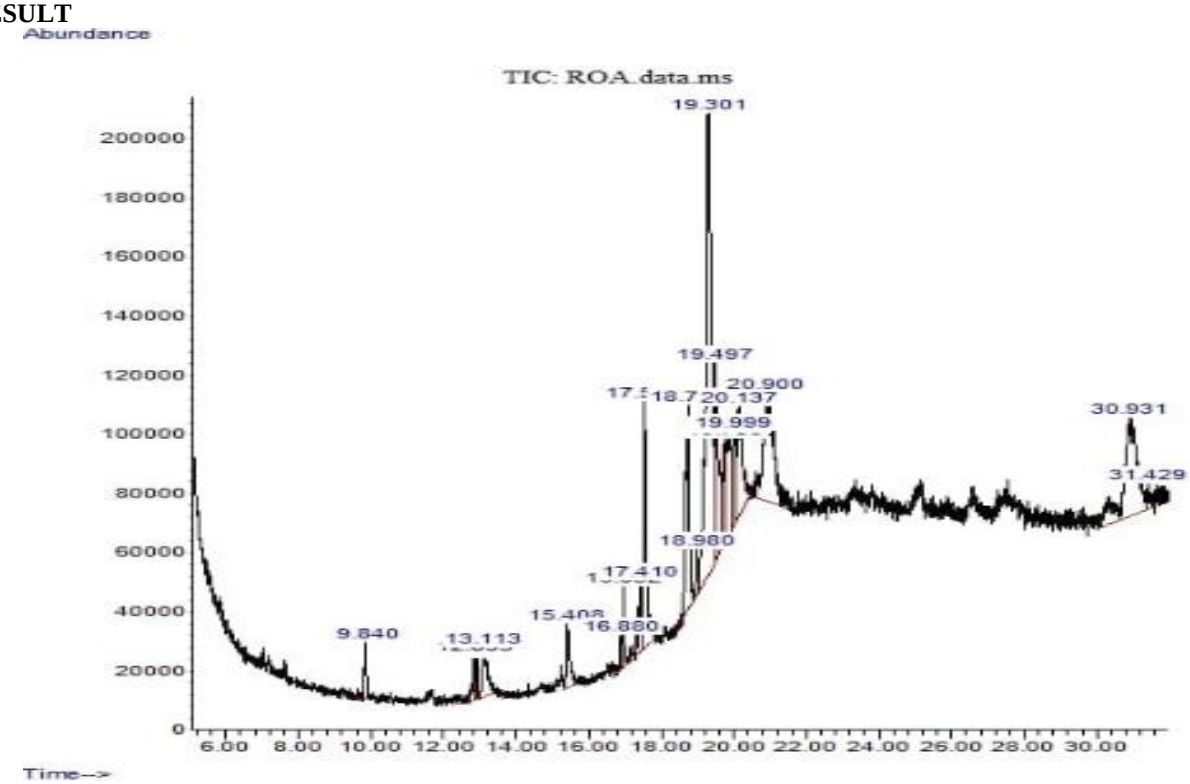

Fig.1: The GC-MS chromatogram of aqueous extract of Salvia rosemarinus plant.

The GC-MS analysis of Salvia rosemarinus aqueous extract revealed a diverse range of compounds, including fatty acids such as n-hexadecanoic acid (2.14% and 7.45%), octadecanoic acid (7.95%), oleic acid (4.25%, 10.94%, and 12.48%), linoleic acid (24.34% as linoelaidic acid and 2.98% as 9,12-Octadecadienoic acid (Z,Z)-), tetradecanoic acid (2.29%), and dodecanoic acid (2.38%), as well as fatty acid esters like hexadecanoic acid, methyl ester (1.90%) and 10-octadecenoic acid, methyl ester (6.38%), with retention times ranging from 9.840 to 31.429 minutes, suggesting the presence of lipids or fatty acid-rich compounds and potential bioactive compounds like oleic acid and linoleic acid.

The GC-FID analysis of aqueous extract of *Salvia rosemarinus* plant

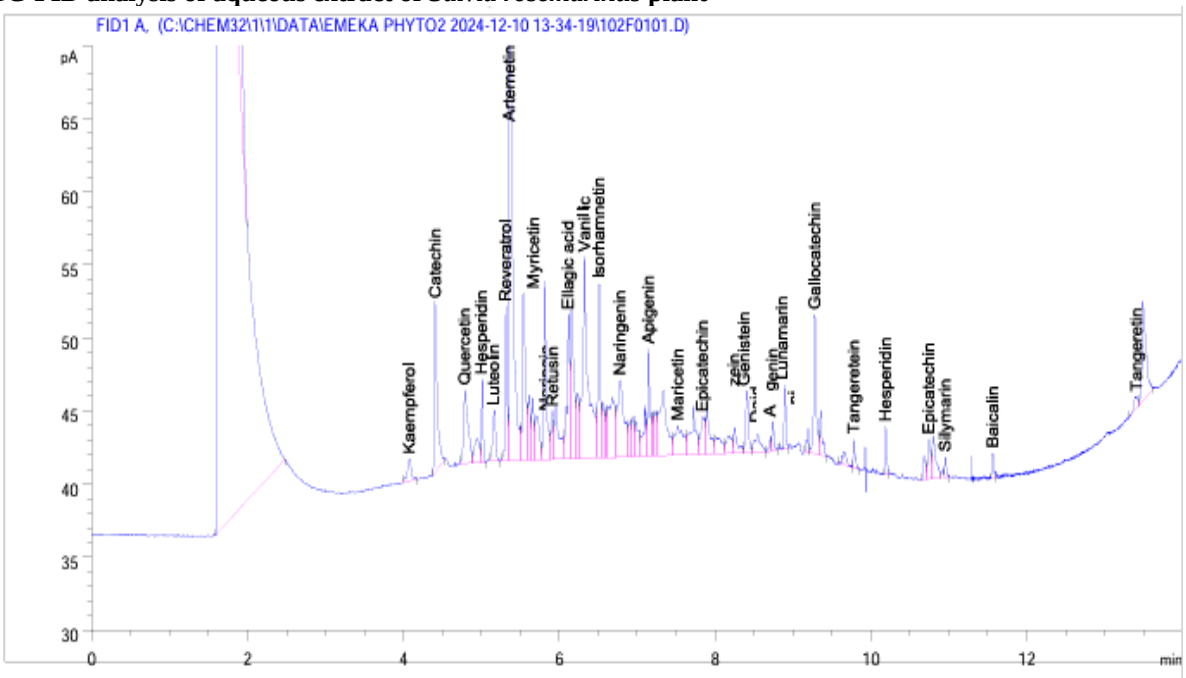

Fig.2: the GC-FID chromatogram of the aqueous extract of salvia rosemarinus plant.

The GC-FID analysis of Salvia rosemarinus aqueous extract revealed the presence of various phytochemicals, including flavonoids such as kaempferol (0.755838 ppm, RT: 4.082), catechin (5.34933 ppm, RT: 4.407), quercetin (3.81463 ppm, RT: 4.789), hesperidin (2.10600 ppm, RT: 5.009 and 0.61448 ppm, RT: 10.187), luteolin (2.24377 ppm, RT: 5.162), myricetin (5.28215 ppm, RT: 5.539), naringin (2.67960 ppm, RT: 5.706), apigenin (2.90527 ppm, RT: 7.145 and 0.444042 ppm, RT: 8.739), and others, as well as stilbenols like resveratrol (3.07204 ppm, RT: 5.318), polyphenols like ellagic acid (3.77163 ppm, RT: 6.117), and isoflavones like genistein (1.49426 ppm, RT: 8.405) and daidzein (0.805419 ppm, RT: 8.248), with a total amount of 84.43349 ppm.

DISCUSSION AND CONCLUSION

This study employed GC-MS and GC-FID analysis, powerful analytical techniques used to identify and quantify phytochemicals in plant extracts (Sparkman et al., 2011; Król et al., 2020; Gupta et al., 2019), to identify phytochemicals in *Salvia rosmarinus* (Rosemary) and investigated its potential sleep-promoting effects in Wistar rats with insomnia. The analysis revealed a diverse array of bioactive compounds, including flavonoids (such as kaempferol, catechin, and quercetin), phenolic acids, and fatty acids, which may contribute to its therapeutic benefits due to their antioxidant and anti-inflammatory properties. These findings are consistent with previous research suggesting that the identification of bioactive compounds in rosemary may provide insights into the development of natural sleep aids (Kruk et al., 2018). Moreover, the sleep-enhancing properties of Rosemary extract have significant implications for human health, potentially leading to novel therapeutic approaches for insomnia and other sleep disorders (Srivastava et al., 2010). Our findings suggest that *Salvia rosmarinus* extract may have potential applications in managing insomnia and sleep-related disorders.

Recommendations

Based on the findings of this study, the following recommendations are made.

1. Further research is needed to confirm the identities and quantities of bioactive compounds present in *Salvia rosmarinus* aqueous extracts.
2. Additional studies should focus on determining the optimal dosage of *Salvia rosmarinus* extract for potential therapeutic use in managing insomnia and sleep-related disorders.
3. Mechanistic studies are required to investigate the sedative and hypnotic effects of *Salvia rosmarinus* extract and its potential therapeutic applications.

REFERENCES

1. Ahmed Z., Abdeslam-Hassan M., Ouassila L., and Danielle B (2020) Extraction and modeling of Algerian Rosemary essential oil using supercritical CO2: effect of pressure and temperature. Terragreen 2020 Clean Energy Solutions for Sustainable Environment (Cesse), 18: 1038–1046.
2. Amaral GP., Mizdal CR., Stefanello ST., Mendez AS.L, Puntel RL., de Campos MMA., Soares FAA., and Fachinetto R (2019) Antibacterial and antioxidant effects of Rosmarinus officinalis L. extract and its fractions. J Tradit Complement Med, 9: 383–392.
3. Bano MJ, Lorente J, Castillo J, Benavente-Garcia O, Marin MP, Del Rio JA, Ortuno A., and Ibarra I (2019) Flavonoid distribution during the

development of leaves, flowers, stems, and roots of Rosmarinus officinalis postulation of a biosynthetic pathway. J Agric Food Chem, 52: 4987–4992.

4. Bjorøy, I., Jørgensen, V. A., Pallesen, S., and Bjorvatn, B. (2020). The prevalence of insomnia subtypes in relation to demographic characteristics, anxiety, depression, alcohol consumption and use of hypnotics. Frontiers in Psychology, 11: 527-529
5. Cheng A-C, Lee M-F, Tsai M-L, Lai C-S, Lee JH, Ho C-T., and Pan M-H (2019) Rosmanol potently induces apoptosis through both the mitochondrial apoptotic pathway and death receptor pathway in human colon adenocarcinoma COLO 205 cells. Food Chem Toxicol, 49: 485–493
6. Gupta, S., Shrivastava, A., & Sharma, R. (2019). Phytochemical analysis of plant extracts using GC-MS and GC-FID. Journal of Pharmaceutical and Biomedical Analysis, 171: 112-121.
7. Khan, M. A., and Al-Jahdali, H. (2023). The consequences of sleep deprivation on cognitive performance. Neurosciences, 28(2): 91–99.
8. Król, E., Wrona, M., & Koczurkiewicz, P. (2020). GC-MS and GC-FID analysis of essential oils and plant extracts. Journal of Essential Oil Research, 32(3): 239-251.
9. Kruk, J., Aboul-Enein, B. H., & Bernstein, J. (2018). Herbal medicines in sleep disorders: A systematic review. Sleep Medicine Reviews, 37: 63-73.
10. Sparkman, O. D., Penton, Z. E., & Kitson, F. G. (2011). Gas chromatography and mass spectrometry: A practical guide. Elsevier
11. Srivastava, J. K., Shankar, E., & Gupta, S. (2010). Chamomile: A herbal medicine of the past with a bright future. Molecular Medicine Reports, 3(6): 895-901
12. Titova, O. E., Lindberg, E., Elmståhl, S., Lind, L., and Benedict, C. (2022). Seasonal variations in sleep duration and sleep complaints: A Swedish cohort study in middle-aged and older individuals. Journal of Sleep Research, 31.
13. Vandekerckhove, M., and Wang, Y. L. (2024). Emotion, emotion regulation and sleep: An intimate relationship. J neuroscience, 5(1): 1–17.