



Aronia Melanocarpa Extract May Modulate Brain Oscillations and Functional Connectivity: Evidence from EEG Analysis

Aronia Melanocarpa Ekstresi Beyin Osilasyonlarını ve Fonksiyonel Bağlantısallığı Modüle Edebilir: EEG Analizinden Kanıtlar

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ABSTRACT

Aim: This study examines the acute and long-term effects of aronia melanocarpa extract (AME) on brain activity using electroencephalography (EEG). The goal is to determine its impact on neural oscillations, functional connectivity, and network efficiency across different frequency bands.

Materials and Methods: Fifteen healthy volunteers (8 males, 7 females) participated in the study. EEG recordings were conducted before the consumption of AME, one-hour post-consumption, and one week after consumption. Power spectral density (PSD), functional connectivity based on the imaginary part of coherence and network efficiency metrics [global efficiency (GE), local efficiency (LE), and transitivity (T)] were analyzed across Delta, Theta, Alpha, Beta I-III, and Gamma I frequency bands.

Results: Acute effects: PSD increased significantly in frontal and temporal regions across multiple frequency bands. Functional connectivity increased, especially in prefrontal-frontal and prefrontal-temporal pathways. Network efficiency was significantly increased in Beta I-III and Gamma bands ($p<0.05$). Long-term effects: one week later, no significant changes in PSD, GE, or T were found. However, LE increased in the left frontal and frontal midline channels ($p<0.05$).

Conclusion: The acute enhancements in PSD and functional connectivity suggest that AME may temporarily boost cognitive functions by promoting neuronal synchronization and network efficiency. The prominent increases in Beta and Gamma bands are consistent with improved attention, memory, and executive functions. However, the lack of sustained effects highlights the need for continuous intake to maintain cognitive benefits. AME acutely enhances brain activity, particularly in Beta and Gamma bands, suggesting improved cognitive processing and neural communication. However, these effects diminish over time, indicating that regular intake may be necessary for sustained cognitive benefits.

Keywords: Aronia extract, electroencephalography, functional connectivity, power spectral density

ÖZ

Amaç: Bu çalışma, aronia melanocarpa ekstresinin (AME) beyin aktivitesi üzerindeki akut ve uzun vadeli etkilerini elektroensefalografi (EEG) kullanarak incelemektedir. Çalışmanın amacı, farklı frekans bantlarında sinirsel osilasyonlar, fonksiyonel bağlantısallık ve ağ verimliliği üzerindeki etkilerini belirlemektir.

Gereç ve Yöntem: Çalışmaya 15 sağlıklı gönüllü (8 erkek, 7 kadın) katılmıştır. EEG kayıtları, AME tüketilmeden önce, tüketimden bir saat sonra ve bir hafta sonra gerçekleştirilmiştir. Güç spektral yoğunluğu (PSD), ve fonksiyonel bağlantısallık [küresel verimlilik (GE), yerel verimlilik (LE) ve geçişlilik (T)] Delta, Teta, Alfa, Beta I-III ve Gama I frekans bantlarında analiz edilmiştir.

Bulgular: Akut etkiler: Birçok frekans bandında, özellikle frontal ve temporal bölgelerde PSD anlamlı şekilde artmıştır. Fonksiyonel bağlantısallık, özellikle prefrontal-frontal ve prefrontal-temporal yollarında artış göstermiştir. Ağ verimliliği, Beta I-III ve Gama bantlarında anlamlı şekilde artmıştır.

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($p<0,05$). Uzun vadeli etkiler: Bir hafta sonra PSD, GE ve T değerlerinde anlamlı bir değişiklik gözlemlenmemiştir. Ancak, sol frontal ve frontal orta hat kanallarında LE artışı tespit edilmiştir ($p<0,05$).

Sonuç: PSD ve fonksiyonel bağlantısallıktaki akut artışlar, AME'nin nöronal senkronizasyonu ve ağ verimliliğini artırarak bilişsel işlevleri geçici olarak güçlendirebileceğini düşündürmektedir. Beta ve Gama bantlarındaki belirgin artışlar, dikkat, hafıza ve yürütücü işlevlerdeki iyileşmelerle tutarlıdır. Ancak, etkilerin kalıcı olmaması, bilişsel faydaların sürdürülebilmesi için düzenli tüketimin gerekli olabileceğini vurgulamaktadır. AME, özellikle Beta ve Gama bantlarında beyin aktivitesini akut olarak artırarak bilişsel işleme ve sinirsel iletişimin iyileştiğini göstermektedir. Ancak, bu etkiler zamanla azalmaktadır ve uzun vadeli bilişsel faydaların sürdürülebilmesi için düzenli tüketim gerekebilir.

Anahtar Kelimeler: Aronia ekstresi, elektroensefalografi, fonksiyonel bağlantısallık, güç spektral yoğunluğu

INTRODUCTION

The healthy functioning of brain activities depends on the balanced operation of neural network dynamics, functional connectivity, and cortical activity. Recent studies have revealed that dietary polyphenols may exhibit neuroprotective effects on the nervous system and improve cognitive functions¹⁻³. Among polyphenols, anthocyanins are particularly known for their antioxidant, anti-inflammatory, and neuroprotective properties against neurodegenerative diseases^{4,5}. By enhancing brain plasticity, they can strengthen synaptic connectivity and regulate neuronal activity.

In this context, aronia melanocarpa extract (AME) stands out as a functional food rich in polyphenols. Due to its polyphenols enriched with anthocyanins, aronia extract has been identified as containing bioactive compounds that may support brain functions⁶. The literature suggests that AME may enhance cognitive performance, reduce neuroinflammation, and exert positive effects on the nervous system through the gut-brain axis⁷. However, studies directly examining the acute and long-term effects of aronia extract on the brain are quite limited.

In this study, the effects of AME consumption on brain activity were evaluated using electroencephalography (EEG). EEG is a non-invasive method that measures the brain's electrical activity through the skull. Its high temporal resolution makes EEG an ideal tool for examining functional connections and frequency components. Since the electrical activity measured in EEG represents a local field potential projected onto the skull, the volume conduction effect may be a limitation for spatial analysis. To mitigate this effect, the Laplacian reference, one of the reference techniques, was applied in functional connectivity analysis. In this study, EEG power spectral density (PSD), and functional connectivity measurements were examined to investigate the effects of aronia extract on neural oscillations and brain network efficiency. The Welch method was used when calculating PSD, and the imaginary part of coherence (iCOH) was used to examine functional connectivity.

The aim of this study is to determine the short and long-term effects of AME on brain activity. The results of this study may contribute to our understanding of the potential effects of dietary polyphenols on brain functions and shed light on the development of neuroprotective strategies.

MATERIALS AND METHODS

Participants and Experimental Procedure

This study included 15 participants, consistent with previous EEG research that often uses 10-20 participants in exploratory neurophysiological studies⁸. The demographic information and clinical characteristics of the participants are presented in Table 1. The research protocol was conducted in accordance with the approvals granted by the Ethics Committee of Nişantaşı University.

Individuals with any physiological or psychiatric conditions that could potentially affect the study outcomes were excluded. Participants were screened to ensure they were not taking any medications that could influence metabolism related to such conditions.

The extract obtained from aronia berries was prepared as 250 mL per serving. EEG recordings were conducted two hours after the participants consumed the aronia berry extract. This timing was adjusted according to the half-life of the compound⁹.

EEG signals were recorded at three different times to assess the acute and long-term effects of the consumption of AME. All of the EEG recordings were performed in a resting state. Participants were seated in a quiet, dimly lit room, with their eyes closed and in a relaxed position. Each session lasted at least 30 minutes to ensure a comprehensive analysis of resting-state brain activity. The first recording was conducted before AME consumption, and the second EEG recording followed two hours later to evaluate the acute effects of AME. During the 2-hour waiting period, participants did not consume any food

Table 1. Demographic and clinical characteristics of participants

Subject number	15
Laterality	93.3% right-handed and 6.6% left-handed
Age (mean \pm SD)	23.93 \pm 1.79
Gender	46.6% women 53.3% men
Literacy rate	100%
SD: Standard deviation	

or beverages other than water, or smoke, and were instructed to follow their usual daily activities. For the assessment of long-term effects, the third EEG recording was performed one week after AME consumption in the same environment.

Ethical permission to conduct this study was approved by the Nişantaşı University Clinical Research Ethics Committee (decision no: 2020/17, date: 25.09.2020). Informed consent was obtained from all participants before inclusion in the study, and the necessary permissions were secured.

Aronia Melanocarpa Extraction

The AME berries originated from Rize, Kırklareli, Türkiye. They began forming in June and were harvested around October and November at their optimal ripeness for processing. After harvest, the berries were transported using vehicles equipped with cold storage facilities. Prior to extraction, they were stored at 4 °C to maintain their quality. The extraction process was conducted within one week without significant delay. Fresh berries were used in this study. AME berries were pureed using a grinder. The resulting pure was mixed with water containing 0.1% hydrochloric acid to form a homogeneous solution. This mixture was extracted using an ultrasonication method at 30 °C for 30 minutes. The ultrasonication process facilitated the release of bioactive compounds by breaking down the cell walls, allowing these compounds to be transferred into the solution.

Following the extraction process, the mixture was centrifuged at 10,000 rpm for 15 minutes. The resulting supernatant (upper liquid phase) was collected and stored at +4 °C to preserve the bioactive compounds.

Total Phenol Analysis

The total phenol content of the aronia fruit extract was determined using the Folin-Ciocalteu method, as described by Chen et al.¹⁰. Briefly, 0.2 mL of the sample (diluted if necessary) was mixed with 1.5 mL of 10% Folin-Ciocalteu reagent and allowed to react for 5 minutes. Then, 1.2 mL of 7.5% (w/v) sodium carbonate (Na_2CO_3) was added and thoroughly mixed. The mixtures were incubated at room temperature for 60 minutes, after which the absorbance was measured at 765 nm using a Shimadzu UV-1800 spectrophotometer against a blank. The analyses were performed in triplicate, and the calibration curve was constructed using gallic acid in the concentration range of 0-250 ppm.

Total Antioxidant Capacity Analysis

The cupric ion reducing antioxidant capacity (CUPRAC) assay was applied to determine the antioxidant capacity of aronia fruit extract, following the procedure described by Apak et al.¹¹. Briefly, 1 mL of 10^{-2} M CuCl_2 , 1 mL of 7.5×10^{-3} M neocuproine, and 1 mL of 1 M NH_4Ac were mixed in a test tube. Then, the

sample (or standard) solution (x mL) and H_2O (1.1 - x mL) were added to the initial mixture to obtain a final volume of 4.1 mL. The mixture was then incubated at room temperature for 30 minutes. Finally, absorbance was measured at 450 nm against a blank using a Shimadzu UV-1800 spectrophotometer (Kyoto, Japan). The calibration curve was constructed using Trolox in the range of 10-100 $\mu\text{mol/L}$.

EEG Recording and Processing

EEG signals were recorded using the 10-20 international electrode placement system, employing 19 EEG channels and mastoid referencing, with ANT Neuro sport EEG device. The recordings were conducted with a sampling frequency of 500 Hz and a finite impulse response band-pass filter applied between 1-45 Hz. For data analysis, PSD were calculated via Welch method¹² for all EEG channels in each participant. Functional connectivity was assessed using the iCOH¹³, while coherence-based network metrics, including global efficiency (GE), local efficiency (LE), and transitivity (T) were evaluated. In functional connectivity analysis, the monopolar reference was replaced with the Laplacian reference¹⁴ through re-referencing to enhance the spatial performance of the analysis. EEG recordings were analyzed across seven frequency bands: Delta (1-4 Hz), Theta (4-8 Hz), Alpha (8-12 Hz), Beta I (12-18 Hz), Beta II (18-24 Hz), Beta III (24-30 Hz), and Gamma I (30-45 Hz), along with the full spectrum (1-45 Hz). These metrics were systematically examined to determine changes in brain network dynamics following AME.

Statistical Analysis

Differences between the initial and post-consumption recordings were statistically analyzed using the Wilcoxon signed-rank test.

Given the exploratory nature of this study and the relatively small sample size, we did not apply a formal correction for multiple comparisons.

A post-hoc power analysis was conducted to evaluate statistical power. Effect sizes (Cohen's d) were computed for each feature and frequency band that showed significant changes. Using G*Power, a paired t-test power analysis was performed with $\alpha=0.05$, $n=15$, and the calculated effect sizes were used to determine statistical power.

RESULTS

The total phenol content of the aronia extract was found to be approximately 800 mg GAE/250 mL (gallic acid equivalents). The total antioxidant capacity of the aronia berry extract was determined using the CUPRAC method. The extract demonstrated a high antioxidant capacity of approximately 1.100 μmol trolox equivalents per liter ($\mu\text{mol TE/L}$).

When comparing the PSD results between the initial recording and the two-hour post-consumption recording, a statistically significant increase in PSD was observed across the average of all EEG channels, as well as in specific brain regions such as the left frontal, right frontal, and right temporoparietal regions for the entire spectrum (1-45 Hz). In the Delta frequency band, PSD increased significantly across all channels, particularly in the left frontal, left temporal, and right temporal regions. A similar increase was observed in the Theta frequency band, with heightened activity in the entire frontal region and the right temporoparietal region. In the Alpha band, PSD values were notably higher in the frontal and right temporal regions, a pattern that was also present in the Beta I band. Additionally, in the Gamma I band, higher PSD values were observed across the entire frontal region.

The iCOH results revealed statistically significant increases in functional connectivity across the whole frequency

spectrum (1-45 Hz). These connections were observed in prefrontal-frontal, interhemispheric frontal, and frontal-central pathways, as well as frontoparietal connections linking anterior and posterior regions. Enhanced connectivity was also present in central-parietal and central-occipital pathways, indicating integration between midline and posterior regions. Furthermore, interhemispheric connectivity was evident in both frontal and occipital regions, while temporoparietal and temporo-occipital interactions extended across lateral areas. These results demonstrated a widespread increase in connectivity, integrating anterior, midline, and posterior regions across both hemispheres. Mentioned connections can be seen in Figure 1A.

No significant connectivity changes were observed in the Delta frequency band given in Figure 1B. However, in the Theta band, stronger connections emerged in the left central-temporal and midline central-occipital pathways, suggesting increased

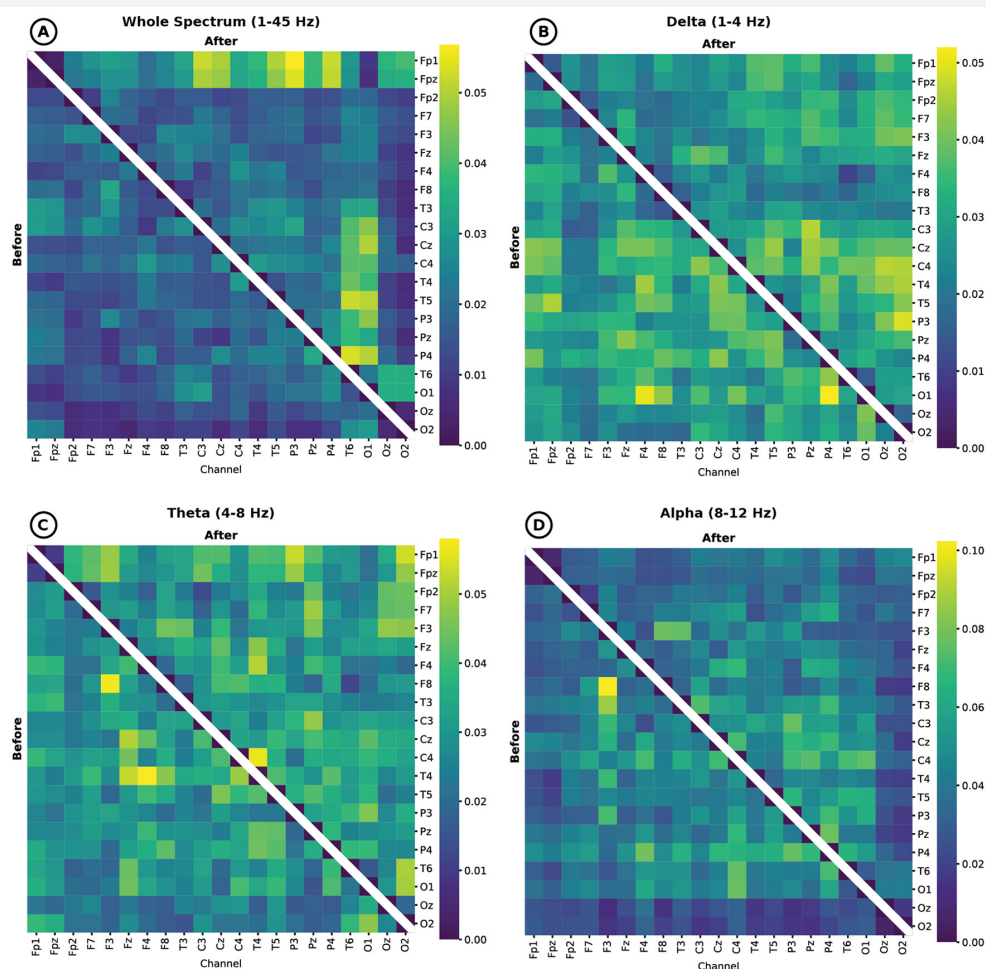


Figure 1. This figure illustrates the statistical comparison of iCOH values between the pre- and 2-hour post-consumption conditions of AME across different frequency bands. Panel A displays the results for the whole frequency spectrum (1-45 Hz), while Panels B, C, and D correspond to the delta (1-4 Hz), theta (4-8 Hz), and alpha (8-12 Hz) bands, respectively

iCOH: Imaginary part of coherence, AME: Aronia melanocarpa extract

integration between these regions. Those connections can be seen in Figure 1C. In the Alpha frequency band, notable connectivity increases were detected in the interhemispheric prefrontal-occipital, right prefrontal-occipital, left central-temporal, midline central-temporal, right temporal-occipital, and interhemispheric occipital pathways, shown in the Figure 1D. These findings indicate enhanced communication across anterior, midline, and posterior regions, as well as between hemispheres. In the Beta I frequency band, stronger connectivity was observed in prefrontal-frontal, prefrontal-parietal, and prefrontal-occipital pathways, demonstrating increased integration between anterior and posterior regions. Frontal-central and frontal-temporal interactions, along with connections extending from temporal and central regions to parietal and occipital areas, also showed increased strength. Interhemispheric occipital connectivity exhibited further enhancement, reinforcing posterior integration. Similar trends were observed in the Beta II frequency band, where stronger

connections were noted in prefrontal-frontal, prefrontal-central, prefrontal-temporal, and prefrontal-occipital pathways, further linking anterior regions with midline and posterior areas. The Beta III frequency band exhibited stronger connectivity across prefrontal, frontal, temporal, parietal, and occipital regions, with pronounced prefrontal-posterior and temporal-occipital interactions. In the Gamma band, significant connectivity increases were observed in prefrontal-temporal, prefrontal-central, and prefrontal-posterior pathways. Frontal-temporal and frontal-posterior connections also displayed enhanced connectivity, alongside stronger interactions in central-posterior, central-occipital, temporal-occipital, and interhemispheric occipital pathways. These connections can be seen in (Figure 2. E, F, G, H), respectively.

When comparing GE before and after AME, a statistically significant increase was observed in the Beta I, Beta II, and Beta III bands (p -value=0.01, 0.02, and 0.02, respectively), as well as in

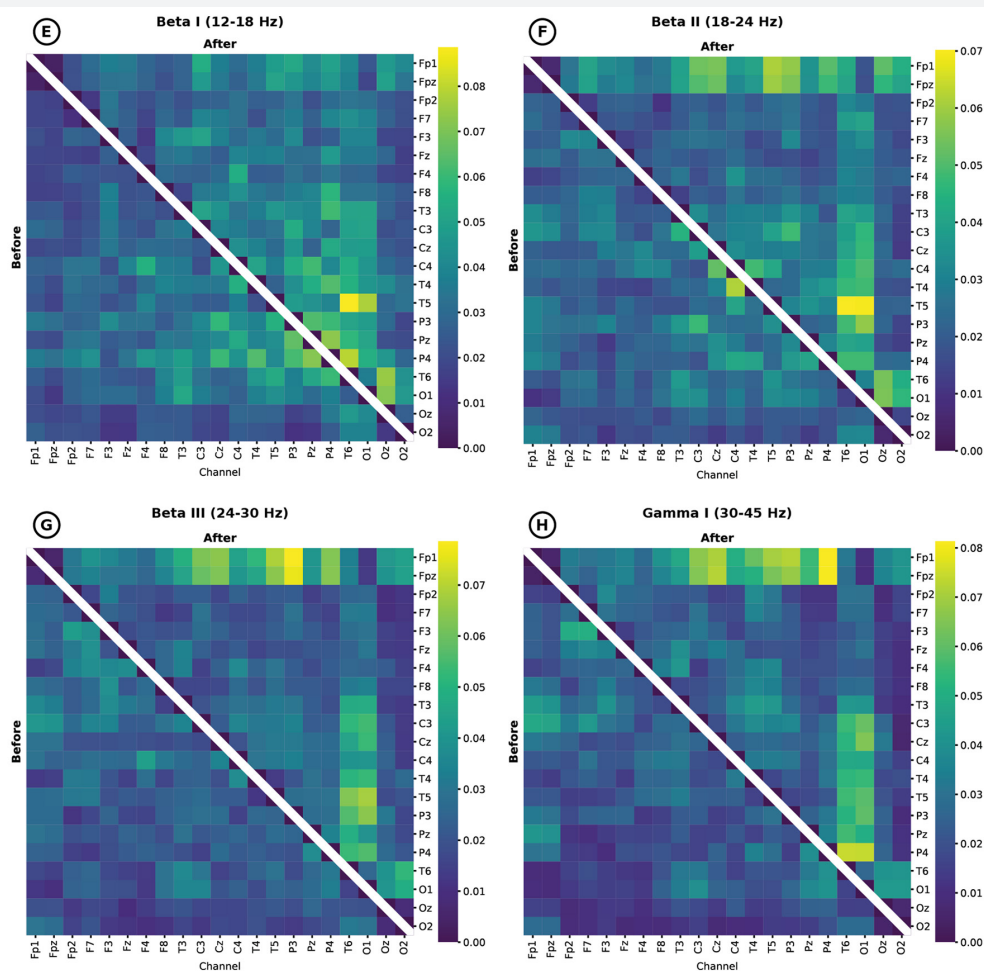


Figure 2. This figure illustrates the statistical comparison of iCOH values between the pre- and 2-hour post-consumption conditions of AME across higher frequency bands. Panels E, F, and G show results for the beta I (12-18 Hz), beta II (18-24 Hz), and beta III (24-30 Hz) bands, respectively, while Panel H presents results for the gamma band (30-45 Hz)

iCOH: Imaginary part of coherence, AME: Aronia melanocarpa extract

the Gamma band (p -value=0.01). A significant increase in GE was also detected across the entire frequency spectrum (1-45 Hz), with a p -value of 0.004. LE showed increases in the prefrontal, left frontal, midline frontal, central, right temporal, right temporoparietal, and occipital regions. Within specific frequency bands, Beta I displayed an increased LE in the same regions, while Beta II showed enhancements in a subset of these regions, excluding the left frontal region. Beta III demonstrated higher LE, particularly in the central and parietal regions, while Gamma activity was associated with increased LE similar to Beta III but without the occipital region. For T, significant increases were observed in Beta I, Beta II, and Beta III (p -value=0.004, 0.03, and 0.04, respectively), as well as in the Gamma band (p -value=0.01). Additionally, significant increases in GE were observed across the entire frequency spectrum, with a p -value of 0.001.

When comparing the PSD results between pre-consumption and one-week post-consumption, although some increases and decreases were observed, they were not statistically significant. Similarly, no significant differences were detected in GE or T. However, LE showed two significant increases in the left frontal and frontal midline channels (p -value=0.05 and 0.03, respectively) within the Beta I frequency band.

Post-hoc power analysis showed effect sizes ranging from approximately 0.82 to 1.17 across different EEG features and frequency bands. The corresponding statistical power ranged between 91.2% and 97.6%, indicating a high probability of detecting significant effects despite the sample size limitation.

DISCUSSION

This study investigated the acute and long-term effects of aronia extract consumption on brain activity using EEG spectral power analysis and functional connectivity measurements. Our findings suggest that aronia extract may modulate neural network dynamics, particularly in the Beta and Gamma frequency bands, which are associated with cognitive processes such as attention, memory, and executive functions. These effects may be closely related to the high polyphenol content and antioxidant capacity of the aronia extract.

Our results revealed a significant increase in PSD in the Delta, Theta, Alpha, Beta I, and Gamma I bands, particularly in the frontal and temporal regions. The total phenolic content of the aronia extract was found to be notably high, approximately 800 mg GAE/250 mL. These findings are consistent with previous studies suggesting that polyphenol-rich dietary interventions can enhance cortical excitability and neuronal synchronization^{15,16}. The increase in Delta and Theta waves in the frontal and temporal regions indicates strengthened resting-state synchronization and cortical inhibition. This enhancement may contribute to improved cognitive stability and greater regularity in resting-state brain activity.

The power increase in Alpha and Beta I bands, particularly in the frontal and right temporal regions, suggests heightened cognitive awareness and readiness. Research indicates that anthocyanins can improve synaptic transmission, thereby enhancing cortical efficiency¹⁷. The increase in Gamma waves in the frontal regions suggests potential improvements in higher-order cognitive functions. Gamma oscillations are closely linked to cognitive integration and memory consolidation¹⁸, suggesting that aronia extract may contribute to complex information processing in the brain.

In addition to EEG power analysis, iCOH-based functional connectivity analyses showed widespread increases in connectivity across the brain. This effect was particularly evident in the strengthened connections between the prefrontal, frontal, temporal, parietal, and occipital regions. The most significant increases in connectivity were observed in the prefrontal-temporal, prefrontal-central, and prefrontal-posterior pathways. These connections play a crucial role in working memory and cognitive control processes^{19,20}. These findings suggest enhanced long-range cortical communication and increased integration capacity of brain networks. Previous studies have proposed that the anthocyanins found in aronia berries support synaptic plasticity, thereby strengthening communication between brain regions⁵⁻¹⁷.

Following aronia extract consumption, significant increases in GE were observed, particularly in the Beta I, Beta II, Beta III, and Gamma bands. GE is a critical measure reflecting the efficiency of brain networks and their information processing capacity²¹. This increase suggests that cognitive processes have become more efficient and that the functionality of neural networks has improved. The enhancement of GE in the Beta and Gamma bands can be associated with faster information processing and improved cognitive performance^{22,23}.

In our study, the high antioxidant capacity of the aronia extract, measured at 1,100 μ mol TE/L, may have played a significant role in reducing oxidative stress. Oxidative stress can cause damage to nerve cells, adversely affecting cognitive functions²⁴. The strong antioxidant capacity of aronia extract may help neutralize free radicals, thereby protecting neuronal health and contributing to the observed increases in both global and local efficiency.

An increase in LE was also detected, particularly in the prefrontal, left frontal, central, right temporal, and occipital regions. The increase in LE in these regions indicates more efficient local neural processing.

T, which measures the density of connections between nodes within a network, is associated with cognitive integration²⁵. The increase in T values in the Beta and Gamma bands indicates that brain networks have become more integrated and better organized.

In EEG analyses conducted one week after aronia extract consumption, less pronounced results were obtained compared to short-term changes. Although some increases and decreases were observed in the PSD analysis, these changes were not statistically significant. No significant changes were detected in GE and T values, suggesting that while aronia extract has strong short-term effects, its long-term effects may be more limited. However, a significant increase in LE was observed in the Beta I band, particularly in the left frontal and midline frontal regions, indicating that some regional adaptations may persist over time.

Study Limitations

Our study was limited to 15 healthy volunteers. This small sample size restricts the generalizability of the results. Studies with larger sample groups are essential for validating these findings. Additionally, only healthy individuals were included in the study. It remains unclear how the effects of aronia extract might differ in individuals with cognitive impairments or neurological disorders. This limitation affects the applicability of the results to clinical populations. Finally, our study focused on a group of young adults with an average age of 23.93 (± 1.79); to enhance the applicability of these findings, future research should extend to other age groups.

It is important to note that the current study provides only a limited scope of the immediate effects of AME, as the evaluation of its long-term effects relies on a single measurement taken one week after consumption, not capturing how these effects change over time. More frequent or extended follow-up assessments would offer a clearer understanding of whether the effects of AME persist over time or gradually diminish with prolonged use.

In this study, EEG was used to assess brain activity through functional connectivity. Although EEG provides high temporal resolution, the spatial limitations of this technique make it challenging to identify the precise sources of brain activity. Even with improvements made through methods such as the Laplacian reference technique, localization remains imprecise. Future studies could benefit from combining EEG with other neuroimaging techniques, such as functional magnetic resonance imaging, to gain a more accurate understanding of the spatial aspects of brain function related to AME consumption.

CONCLUSION

This study has demonstrated that AME acutely enhances brain activity, particularly by increasing PSD and functional connectivity in the Beta and Gamma frequency bands. These enhancements suggest improvements in neuronal communication and cognitive processing. Additionally, significant increases in network efficiency, especially in

the Beta I-III and Gamma bands, were observed, indicating a potential for faster information processing and better cognitive performance.

However, these effects were not sustained over the long term, as no significant changes were observed in GE and T one week after consumption. Nevertheless, a persistent increase in LE was detected in the left frontal and midline frontal regions, suggesting that some regional neural adaptations may continue over time.

Overall, the findings indicate that aronia extract has the potential to modulate neurological activity in the short term, but regular consumption may be required to achieve long-term benefits. These results support the potential role of polyphenol and antioxidant-rich dietary interventions in enhancing cognitive health and highlight the need for larger-scale, long-term studies to further explore these effects.

Ethics

Ethics Committee Approval: Ethical permission to conduct this study was approved by the Nişantaşı University Clinical Research Ethics Committee (decision no: 2020/17, date: 25.09.2020).

Informed Consent: Informed consent was obtained from all participants before inclusion in the study, and the necessary permissions were secured.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.M.K., İ.K., Concept: A.M.K., İ.K., Design: A.M.K., İ.K., Data Collection or Processing: A.M.K., A.Ö., M.Y.Ö., İ.K., Analysis or Interpretation: A.M.K., A.Ö., M.Y.Ö., Literature Search: A.M.K., A.Ö., M.Y.Ö., İ.K., Writing: A.M.K., İ.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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