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Modulatory effects of Kratom extract on the gut microbiota of rats: implications for health

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Abstract

Background Kratom (*Mitragyna speciosa*), a plant native to Southeast Asia, is commonly used as a supplement for fatigue, pain relief, mood enhancement, and euphoria. Kratom extract exhibits diverse pharmacological properties, including antioxidant, anti-inflammatory, and gastrointestinal effects, with studies showing its ability to modulate gut microbiota and stimulate beneficial bacteria growth. Given these properties, kratom treatment may produce significant effects in a rat model, warranting further investigation.

Methods Male Wistar rats were administered kratom extract orally on a daily basis for 28 days. Fresh fecal samples were collected and analyzed for changes in gut microbiome composition using 16S rRNA sequencing. Hematological parameters and lipid profiles were also measured to evaluate any systemic effects.

Results The administration of kratom extract did not significantly affect hematological parameters or lipid profiles. However, notable changes were observed in gut microbiota composition, with significant increases in specific bacteria such as *Candidatus Stoquefichus* and *Prevotellaceae UCG-001*, and a decrease in *Corynebacterium*. LEfSe and cladogram analyses corroborated the higher prevalence of *Candidatus Stoquefichus*, *Prevotellaceae UCG-001*, and *Erysipelatoclostridiaceae* in the kratom treatment group compared to controls.

Conclusions Kratom extract significantly alters gut microbiome composition in rats, promoting beneficial bacteria while also elevating certain taxa associated with negative health outcomes. These mixed effects highlight the need for further research on the long-term implications of kratom use for gut health and its broader health consequences, as well as potential therapeutic applications.

Clinical trial number Not applicable.

Keywords Kratom extract, *Mitragyna speciosa*, Gut microbiota, Microbial community analysis

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Introduction

Kratom (*Mitragyna speciosa*) is a native plant in Southeast Asia that grows naturally in several countries including Thailand, Indonesia and Malaysia [1]. Kratom can interact with serotonergic (5-HT) and μ-opioid receptors, which contributes to its desirable psychotropic and pain-relieving effects. As a result, it is often utilized as a supplement for fatigue, pain relief, mood enhancement, and euphoria [2]. However, the major side effects of kratom have been reported which are hepatotoxicity and withdrawal [3]. Moreover, prolong used of kratom-containing herbal supplements caused a mixed hepatocellular and cholestatic pattern of acute liver injury, acute kidney injury, and pancolitis [3]. In Thailand, especially in the southern region, fresh kratom leaves are commonly chewed or boiled to create a decoction, while dried leaves can be smoked, brewed into tea, or consumed as an herbal beverage [4, 5]. The Office of the Narcotics Control Board (ONCB) states that kratom decriminalization in Thailand represents 'partial legalization.' This allows for its use in medical applications, as a dietary supplement, for research purposes, and for personal or traditional use in accordance with cultural rights [4].

Kratom extract has been reported to exhibit various pharmacological properties, including antioxidant, anti-inflammatory, antibacterial, antiproliferative, and anti-analgesic effects, as observed in both in vitro and in vivo studies [6–9]. These diverse effects are primarily attributed to the alkaloids and polyphenols present in kratom, which have been shown to interact with various biological pathways. For instance, studies have demonstrated that mitragynine, one of the main alkaloids, possesses anti-inflammatory properties by inhibiting the production of pro-inflammatory cytokines [9]. Kratom appears to exert effects on the gastrointestinal system as well [6]. The methanolic extract of kratom leaves demonstrated in vivo effects in rats by reducing defecation frequency and fecal weight in a model of castor oil-induced diarrhea. While a single dose of the extract decreased intestinal transit, prolonged intake did not lead to further reductions. Additionally, pre-treatment with naloxone did not influence defecation frequency, suggesting that kratom may act through pathways beyond opioid receptors [10]. Kratom extract exhibits both acute and chronic effects, including a reduction in food and water intake [11]. Mitragynine, when injected into the lateral cerebroventricle, had no effect on acid secretion. Its anorectic and weight-loss effects may be associated with the direct inhibition of neurons in the lateral hypothalamus [12]. Recent research has highlighted the effects of kratom extracts on gut microbiota and metabolite production in fecal batch culture [13]. In particular, water-soluble kratom extracts have been shown to modulate the composition of gut microbiota, stimulating the growth of beneficial bacteria such as *Bifidobacterium*. This modulation can have significant implications for gut health, as a balanced microbiota is essential for optimal metabolic function and immune response. Furthermore, the metabolites produced during fermentation, such as short-chain fatty acids, play crucial roles in maintaining gut integrity and influencing systemic inflammation. Given these pharmacological properties and their potential impact on gut health, it is reasonable to expect that kratom treatment may yield some effects in a rat model.

The gut microbiome, consisting of bacteria, archaea, viruses, and eukaryotic microbes, primarily resides in the human intestines and includes around 1,000 different species [14]. The major phyla that make up the gut microbial community are Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia [15]. Gut dysbiosis, characterized by reduced microbiota diversity, a decline in beneficial microbes, or an overgrowth of harmful ones, can result from host-specific factors such as genetic background and lifestyle habits, as well as environmental factors like diet and medication [16]. Several studies have shown that treating rats with herbal extracts can impact the composition of their intestinal microflora [17].

The gut microbiota significantly influences both the physiology and pathophysiology of the brain, due to the bidirectional interactions between the intestine and the nervous system [14]. Gut dysbiosis is associated with abnormal brain protein aggregation, inflammation, and immune dysregulation, as evidenced by both animal and human studies [18]. The interaction between the central nervous system (CNS) and gut microbiota occurs through various gut-brain axis mechanisms, including direct and indirect pathways such as the immune, neuroactive, neural, and endocrine pathways [19]. Microbes can produce various neuroactive compounds directly and also stimulate the host to generate additional metabolites and neurotransmitters [20]. The intestinal microbiota produces key neurotransmitters, including dopamine, serotonin, norepinephrine, glycine, and gamma-aminobutyric acid. Imbalances in these neurotransmitters may contribute to various neurological disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), autism spectrum disorder (ASD), anxiety disorders, and depression [21]. Despite these insights, the effects of kratom extract on gut microbiota, gut histology, and systemic hematological parameters and lipid profiles have yet to be explored. This study aims to fill this gap by assessing the impact of kratom extract on these aspects in an animal model. The findings may provide valuable information regarding the potential applications of kratom in preventing or treating neurological disorders, while also raising concerns about the adverse effects associated with long-term use.

Materials and methods

Animals and experimental design

Twelve male Wistar rats, aged five weeks, were procured from Siam Nomura International Co., Ltd. in Bangkok, Thailand. They were housed in pairs in the Animal Laboratory Center at Thammasat University under standard environmental conditions: temperature 22 ± 1 °C, relative humidity 30–70%, light 130–325 lx, dark/light cycle 12/12 hours, noise < 86 dB. A one-week acclimatization period preceded the experiments. The rats were given access to standard chow diet and reverse osmosis water *ad libitum* [17]. Body weight (BW) and food intake were recorded at baseline and subsequently on a weekly basis throughout the study. All procedures adhered to the NIH Guiding Principles in the Care and Use of Animals and received approval from the Thammasat University Animal Care and Use Committee (Protocol No. 018/2022). A schematic of the experimental design is presented in Fig. 1.

Preparation of Kratom leaf extract and animal administration

Kratom leaves were collected from Nakhon Si Thammarat Province, Thailand. The formal identification of the plant material used in this study was undertaken by Dr. Nattaya Thongsepee. The voucher specimen was stored in the herbarium of the Research Unit in Nutraceuticals and Food Safety, Thammasat University, Pathum Thani, Thailand. The deposition number is 2022004. The extraction procedure was carried out as follows: (1) The fresh kratom leaves (2 kg) were washed, dried, and ground; (2) The kratom powder was boiled in water for 30 min, after

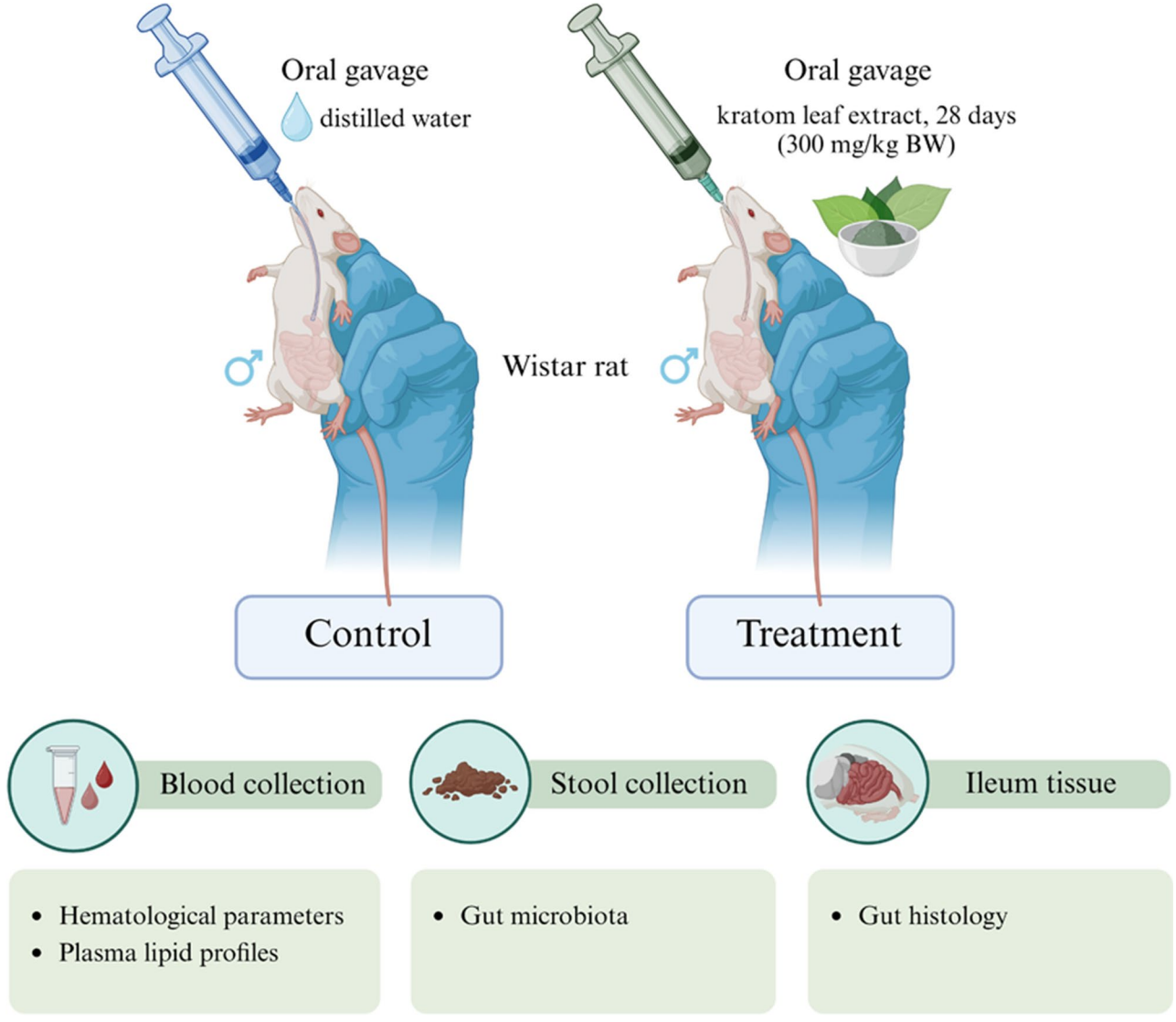

Fig. 1 Experimental Design Schematic. Schematic representation of the experimental design for this study, outlining the treatment regimen and sampling protocol for kratom extract and control groups

which the aqueous extract was filtered using a colander and Whatman filter paper; (3) The water was removed from the solution using a rotary evaporator, and the concentrated solution was further dried using a freeze dryer to yield 80 g of a brown solid extract. The solid extract was stored at -20 °C until use. The chemical composition of the kratom extract was analyzed using high-performance liquid chromatography (HPLC, Agilent Technologies, Germany) by the Office of Scientific Instrument and Testing, Prince of Songkla University, Songkhla, Thailand. Detailed information regarding the HPLC chromatograms is provided in the supplementary data 1.

For animal administration, the kratom extract was dissolved in distilled water to achieve a concentration of 120 mg/mL. The kratom solution was stored at 4 °C until use. The aqueous extract was administered via oral gavage at a dose of 300 mg/kg BW daily for 28 days, while the control group received distilled water. The treatment dose was based on the effective dose of kratom aqueous extract for alleviating ethanol withdrawal-induced behaviors in rodents [22].

Investigation of hematological parameters and plasma lipid profile

After 28 days of kratom treatment, the rats were anesthetized using isoflurane inhalation (5% for induction and 1% for maintenance). The jugular vein and carotid artery were cannulated with polyethylene tubing filled with 0.9% NaCl solution for fluid perfusion and blood sampling, respectively. The hematological parameters, including red blood cell count (RBC), white blood cell count (WBC), hemoglobin (Hb), hematocrit, mean cell volume, mean cell hemoglobin, mean cell Hb concentration, red blood cell distribution width (RDW), mean platelet volume, platelet count, and counts of neutrophils, lymphocytes, monocytes, eosinophils, and basophils, were analyzed. Additionally, blood samples from the rats were used to determine plasma lipid profiles, including levels of cholesterol, triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). The hematological parameters and plasma lipid profiles were analyzed at the Health Care Service Center, Faculty of Allied Health Sciences, Thammasat University, Thailand.

Collection of stool and ileum tissue samples

For euthanasia, the 0.1 M phosphate-buffered saline was perfused via the jugular vein, and the portal vein was cut for the drainage of the perfused fluid. The animals were confirmed dead through established procedures, which included verifying the absence of heartbeat and respiration, as well as a lack of response to stimuli. Subsequently, fresh fecal samples were collected from the colon of each rat using aseptic techniques and stored at − 80 °C until analysis. Additionally, ileum tissue samples were collected from each rat and fixed in 4% paraformaldehyde for histological examination [23].

Histological observation

To evaluate the effect of kratom extract on the colonic mucosa of rats, histological experiments were conducted using ileum tissue from both control and treatment groups ($n = 5$ each). The tissues were processed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin. A licensed pathologist assessed the morphology of the tissue samples under blinded conditions. Images of the tissue sections were captured using a Nikon ECLIPSE Ci microscope coupled with a Nikon DS-Fi2 camera (Nikon Instruments Inc., Tokyo, Japan).

DNA extraction

Fresh fecal samples were collected from the rats' colon post-sacrifice using aseptic techniques and stored at − 80 °C until analysis. Metagenomic DNA from prokaryotes was isolated from 0.25 g of stool samples using the QIAamp PowerFecal Pro DNA Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quality of the DNA samples was assessed using a DeNovix Fluorometer (Wilmington, DE, USA).

16S rRNA library sequencing

The prokaryotic 16S rRNA gene targeting the V3–V4 region was amplified utilizing the Qiagen QIAseq 16S/ITS Region panel (Qiagen, Hilden, Germany). The specific primers used in this study are Forward Primer: 5′- TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3′ and Reverse Primer: 5′- GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′. Different sequencing adaptors were added to the 16S rRNA amplicons using the QIAseq 16S/ITS Region Panel Sample Index PCR Reaction (Qiagen, Hilden, Germany). The quality and concentration of the DNA libraries were evaluated with a DeNovix QFX Fluorometer and a QIAxcel Advanced system (Qiagen, Hilden, Germany). Finally, sequencing of the 16S rRNA libraries was performed on an Illumina MiSeq600 platform (Illumina, San Diego, CA, USA).

Bioinformatics analyses

The raw sequences were sorted into groups according to their 5′ barcode sequences. Sequence processing was conducted using the DADA2 v1.16.0 pipeline (https://benjjneb.github.io/dada2/, accessed on 5 March 2024). This pipeline identifies microbial diversity and community structures by analyzing unique amplicon sequence variants (ASVs) [24]. Microbial taxa were classified using Silva version 138 as the reference database [25]. The

Table 1 Body weight

	Control (*n* = 6)	Kratom (*n* = 6)	Significance
Begin of experiment, g	294 ± 4	307 ± 5	ns
The end of experiment, g	424 ± 6	440 ± 10	ns
Body weight change, g	106 ± 2	110 ± 8	ns

Data were represented as mean ± SEM. ns = non-significance

alpha diversity indices, including Chao1 richness, Shannon, and PD whole tree, were calculated with DADA2 software. For beta diversity analysis, non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity and principal coordinate analysis (PCoA) were visualized using Phyloseq data. To identify bacterial biomarkers, linear discriminant analysis effect size (LEfSe) and cladogram plots were employed. The raw data were uploaded to the NCBI SRA database, under the BioProject ID: PRJNA1166840.

Data analysis

Alpha diversity was assessed through pairwise comparisons of observed ASVs, Chao1, Shannon, and PD whole tree metrics using the Kruskal–Wallis test, with a significance threshold of $p < 0.05$. To assess significant differences in beta diversity among groups, a permutational multivariate analysis of variance (PERMANOVA) was performed at the same significance level. Additionally, the Kruskal–Wallis sum-rank test was applied in LEfSe analysis to identify bacterial biomarkers that showed significant differences in abundance between the sample groups.

Statistical analysis

All statistical analyses were conducted using GraphPad Prism 8 (San Diego, CA, USA), with P-values below 0.05 considered statistically significant. All data were presented as the mean ± SEM. Mann–Whitney tests were conducted to compare the percentage relative abundance between the control and treatment groups.

Results

Effect of Kratom extract on body weight, food intake, hematological parameters and plasma lipid profiles

The effect of kratom extract on body weight and food intake in the experimental animals was determined. The rats appeared normal and healthy throughout the study.

Table 3 Hematologic parameter of rats

Parameters	Control (*n* = 6)	Kratom (*n* = 6)	Significance
RBC (x10^6 /μL)	7.1 ± 0.2	7.5 ± 0.1	ns
WBC (x10^3 /μL)	8.3 ± 1.1	9.3 ± 0.6	ns
Hemoglobin (Hb; g/dL)	13.5 ± 0.5	14.6 ± 0.3	ns
Hematocrit (%)	36.8 ± 1.3	39.4 ± 0.9	ns
Mean cell volume (fL)	52.1 ± 0.6	52.4 ± 0.5	ns
Mean cell hemoglobin (pg)	19.1 ± 0.2	19.5 ± 0.2	ns
Mean cell Hb Concentration (g/dL)	36.7 ± 0.1	37.1 ± 0.2	ns
RDW (%)	13.0 ± 0.2	13.0 ± 0.2	ns
Mean platelet volume (fL)	7.0 ± 0.0	6.8 ± 0.2	ns
Platelet count (x10^3 /μL)	452 ± 85	604 ± 46	ns
Neutrophils (%)	55.9 ± 7.0	53.5 ± 5.6	ns
Lymphocytes (%)	52.0 ± 10.9	45.9 ± 5.6	ns
Monocytes (%)	1.1 ± 0.5	0.4 ± 0.1	ns
Eosinophils (%)	0.1 ± 0.0	0.2 ± 0.1	ns
Basophil (%)	0.0 ± 0.0	0.0 ± 0.0	ns

Data were represented as mean ± SEM. ns = non-significance

Table 4 Plasma lipid profiles

Parameters	Control (*n* = 6)	Kratom (*n* = 6)	Significance
LDL-direct (mg/dL)	10.7 ± 0.8	8.5 ± 1.06	ns
HDL (mg/dL)	30.0 ± 1.9	30.8 ± 2.1	ns
Cholesterol (mg/dL)	47.3 ± 3.4	43.8 ± 2.7	ns
Triglyceride (mg/dL)	99.3 ± 36.7	130 ± 36.0	ns

Data were represented as mean ± SEM. ns = non-significance

There was no difference in body weight between groups at the beginning of the study and after 28 days of treatment with kratom extract (Table 1). Furthermore, no significant difference in food consumption was observed between the groups during the treatment period (Table 2).

Measured hematological parameters of the rats are presented in Table 3. The values for hematologic parameters in the control and treatment groups were closely similar. Based on statistical comparisons, there was no statistically significant difference ($p < 0.05$). Plasma lipid profile measurements also did not reveal a statistically significant difference between the control and treatment groups (Table 4). However, triglyceride values displayed individual variations in both groups.

Effects of Kratom extract on intestinal histopathology

No significant alterations in the architecture of the colonic mucosa of the rats were observed following

Table 2 Food intake

Food intake (g)	Before experiment	Week (s) after oral gavage of Kratom				Significance
		1	2	3	4	
Control (*n* = 6)	21.2 ± 0.5	21.2 ± 0.3	21.4 ± 1.0	21.7 ± 0.4	21.7 ± 1.0	ns
Kratom (*n* = 6)	22.0 ± 0.6	21.9 ± 0.5	22.3 ± 0.5	22.2 ± 0.7	22.8 ± 0.8	ns

Data were represented as mean ± SEM. ns = non-significance

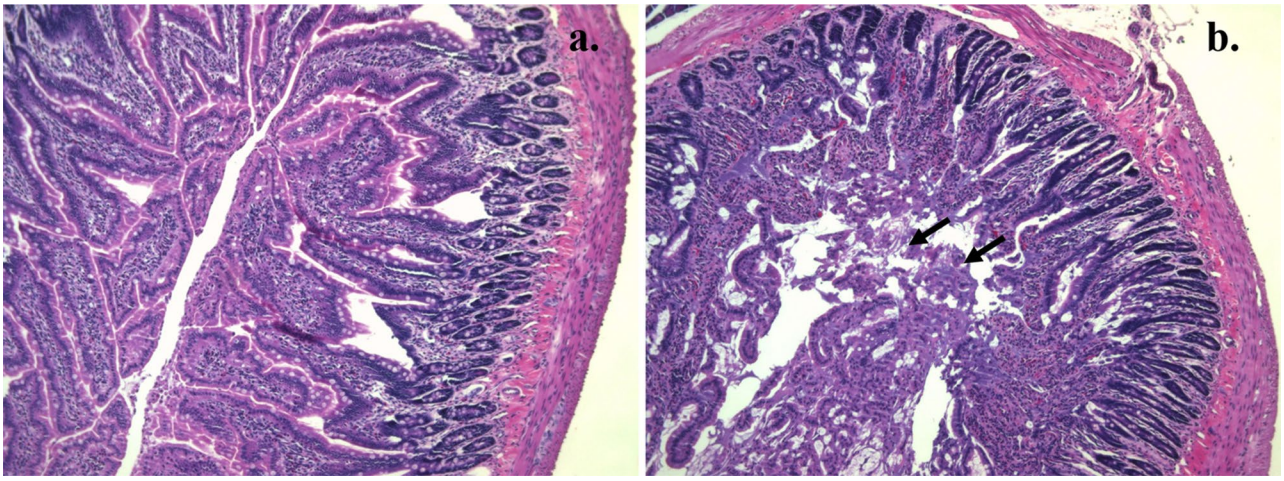

Fig. 2 Histological Appearance of Colonic Mucosa. Histological images of the colonic mucosa from rats in the control (**a**) and kratom treatment (**b**) groups, observed under 10× magnification. Arrows indicate focal autolytic changes in the kratom-treated group

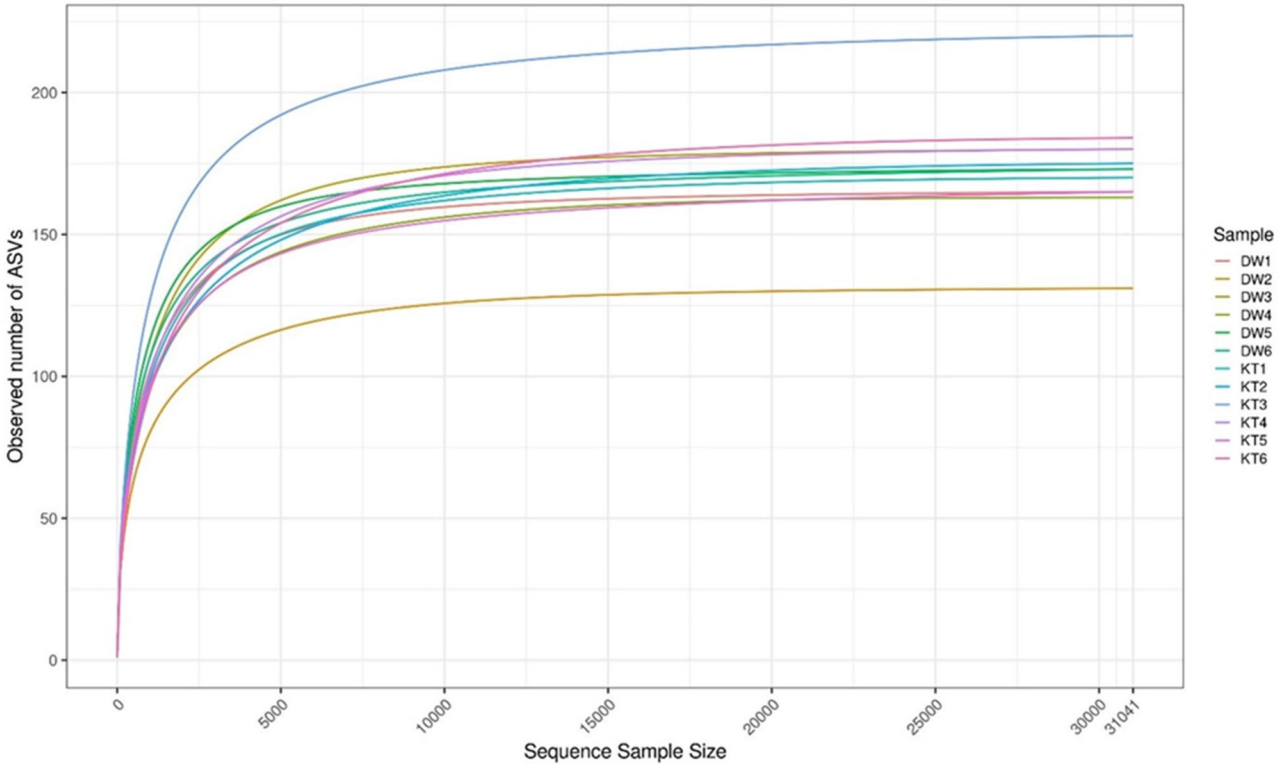

Fig. 3 Rarefaction Curve for Species Richness. Rarefaction curves depicting species richness within and between sequencing reads for gut microbiota samples from control and kratom-treated rats

treatment with kratom (Fig. 2). Five samples from each group were examined, and normal intestinal mucosa appearances were found in all samples except for one from the kratom treatment group, which showed focal autolytic changes (Fig. 2b).

Effects of Kratom extract on the intestinal flora of rats

Changes in flora structure

A 16S rRNA gene-based analysis was used to assess bacterial communities in the rat fecal samples, with six replicates from each group. After processing, a total of 589,954 high-quality 16S rRNA reads were obtained. As shown in Fig. 3, the rarefaction curve represents species richness (the number of different species) within and between sequencing reads. It can be used to estimate the

number of amplicon sequence variants (ASVs) or taxa present within the same size of reads [26]. The approximate saturation of microbial richness for all samples was reached at a sequencing depth of 31,041, as indicated by the rarefaction curves. A plateau in the curve was observed when approximately 15,000 sequencing depths were reached. This finding adequately estimated the true bacterial compositions of the gut microbiome among the sample groups. The number of observed ASVs in KT3 was the highest, with 220 ASVs, suggesting that the abundance of microbiota in this sample was greater compared to the others. The DW2 had a lower count, with approximately 131 ASVs. However, there was no significant difference in the number of observed ASVs between the control and treatment groups.

The alpha diversity index (α-diversity) provides a statistical description of community diversity. The diversity index was calculated using DADA2 software, and the results are presented in Table 5 and Fig. 4. The observed abundance of ASVs in the treatment group was similar to that in the control group. This result was consistent with the rarefaction curves from the previous data. Additionally, similar findings were noted for Chao1 (bacterial richness), Shannon (bacterial diversity), and phylogenetic diversity (PD) in the whole tree. The results indicated that there was no statistically significant difference between the treatment and control groups (Kruskal-Wallis test; $p > 0.05$). This finding suggests that bacterial abundance and diversity were comparable between the treatment and control groups.

Beta-diversity (β-diversity) analysis was used to compare the magnitude of differences among samples in terms of species diversity. In this experiment, the fecal flora diversity data from rats in all treatment groups were analyzed using principal coordinate analysis (PCoA) based on weighted and unweighted UniFrac distances, generalized UniFrac (GUniFrac) distances, and non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity (Fig. 5). Both the weighted and unweighted UniFrac PCoA, as well as NMDS based on Bray-Curtis distance, indicated that microbiota communities were closely aligned between the treatment and control groups (PERMANOVA test; $p = 0.8651$, 0.1089, and 0.2328, respectively). This finding suggests that the microbiota structures in the treatment group were relatively similar to those in the control group.

Characteristic changes in intestinal flora

The microbiota composition at the phylum, family, and genus levels in both the control and treatment groups was identified and illustrated using bar charts (Fig. 6).

At the phylum level, a total of six different bacterial phyla were identified among the top 100 taxonomic classifications in both the control and treatment groups. Firmicutes were the most prevalent, accounting for an average of 94.4 ± 0.45%, followed by Bacteroidota and Actinobacteriota. The relative abundance of all phyla in the treatment group was similar to that in the control group.

A total of 30 different bacterial families were identified in both the control and kratom treatment groups in this study. Analysis of the bacterial community structure at the family level revealed that *Lachnospiraceae*, *Peptostreptococcaceae*, *Ruminococcaceae*, *Lactobacillaceae*, *Erysipelotrichaceae*, *Monoglobaceae*, and *Butyricicoccaceae* were the most dominant in the fecal samples. The relative abundance of *Corynebacteriaceae* was significantly decreased in the treatment group compared to the control group ($p < 0.05$). Conversely, a significant increase in the abundance of *Erysipelatoclostridiaceae* was observed in the treatment group compared to the control group ($p < 0.05$).

At the genus level, a total of 68 genera were detected among the samples (Fig. 6). The major genera identified included *Romboutsia*, *Ruminococcus*, *Lactobacillus*, *Dorea*, *Turicibacter*, *Monoglobus*, and *Blautia*. Treatment

Table 5 Estimated sequencing coverage and alpha diversity indices of the bacterial taxonomic profiles of the 16S rRNA gene sequences at the genus level

Group	Sample ID	Raw Reads	Observed ASVs	Chao1	Shannon	PD_whole_tree
Control	C1	35,403	165	165.3333	3.590757	25.15591
	C2	31,041	131	131.5	3.396861	23.37625
	C3	45,376	180	180.25	3.569521	26.21777
	C4	47,369	163	163	3.376287	24.98278
	C5	40,257	173	173.2	3.841324	25.10661
	C6	48,081	173	175.5	3.585396	24.26468
KT	KT1	52,911	170	170.1667	3.503344	25.61381
	KT2	57,841	175	175.4286	3.592804	26.51206
	KT3	58,447	220	221.1111	3.794813	28.18387
	KT4	56,875	180	180.125	3.535289	26.42536
	KT5	53,542	165	167.5	3.650316	25.52932
	KT6	62,811	184	184.4286	3.295167	26.1718

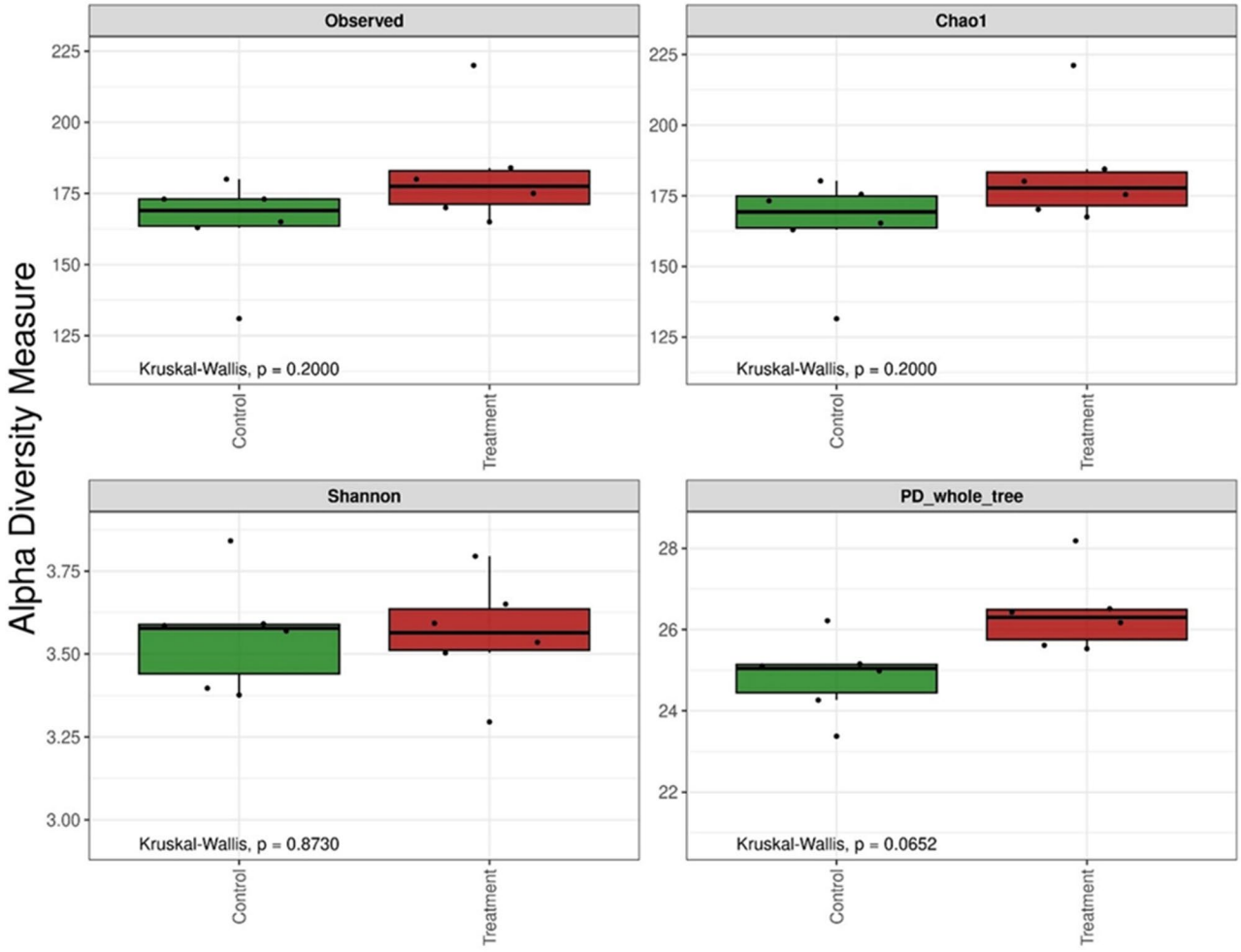

Fig. 4 Alpha Diversity Metrics. Box plots showing alpha diversity measures (observed number of ASVs, Chao1, Shannon index, and PD whole tree) for control (green bars) and kratom treatment (red bars) groups. Black dots represent individual samples within each group

with kratom extract significantly increased the abundance of *Candidatus Stoquefichus* and *Prevotellaceae UCG-001*, while it reduced the abundance of *Corynebacterium* compared to the control group ($p < 0.05$) (Fig. 7). This finding suggests that kratom treatment influenced the composition of certain microbes in the gut of the rats.

Linear discriminant analysis effect size (LEfSe) was employed to identify biomarkers between the two groups based on bacterial relative abundances. Bacterial taxa with LDA scores greater than 2 are shown in Fig. 8. Additionally, a cladogram illustrating the differentially abundant taxonomic clades according to the LEfSe analysis is presented in Fig. 9. Bacteria in the classes *Actinobacteria*, *Corynebacteriales*, and *Corynebacteriaceae*, as well as the genus *Corynebacterium*, were identified as core gut microbiota in the control group ($p < 0.05$). In contrast, *Erysipelatoclostridiaceae*, *CandidatusStoquefichus*, and *PrevotellaceaeUCG-001* were highly prevalent in the treatment group ($p < 0.05$).

Discussion

Kratom (*Mitragyna speciosa*) has become a subject of interest due to its possible benefits for pain relief, mood improvement, and its varying effects as either a stimulant or sedative, influenced by dosage. Kratom extract, primarily composed of mitragynine, has been shown to exhibit various pharmacological effects both in vitro and in vivo [6–8]. Nonetheless, its safety, especially concerning toxicity, requires thorough investigation. Therefore, this study aimed to assess the effects of kratom extract on hematological parameters, lipid profiles, and the gut microbiome in rats.

Treatment of Sprague Dawley rats with kratom decoction (150 mg/kg) for 28 days revealed no significant changes in body weight or hematological profile, except for a reduced platelet count [27]. In our study, treating Wistar rats with kratom extract (300 mg/kg) for 28 days also had no effects on body weight or hematological parameters. Overall, the results align with previous findings, except that platelet counts showed no significant

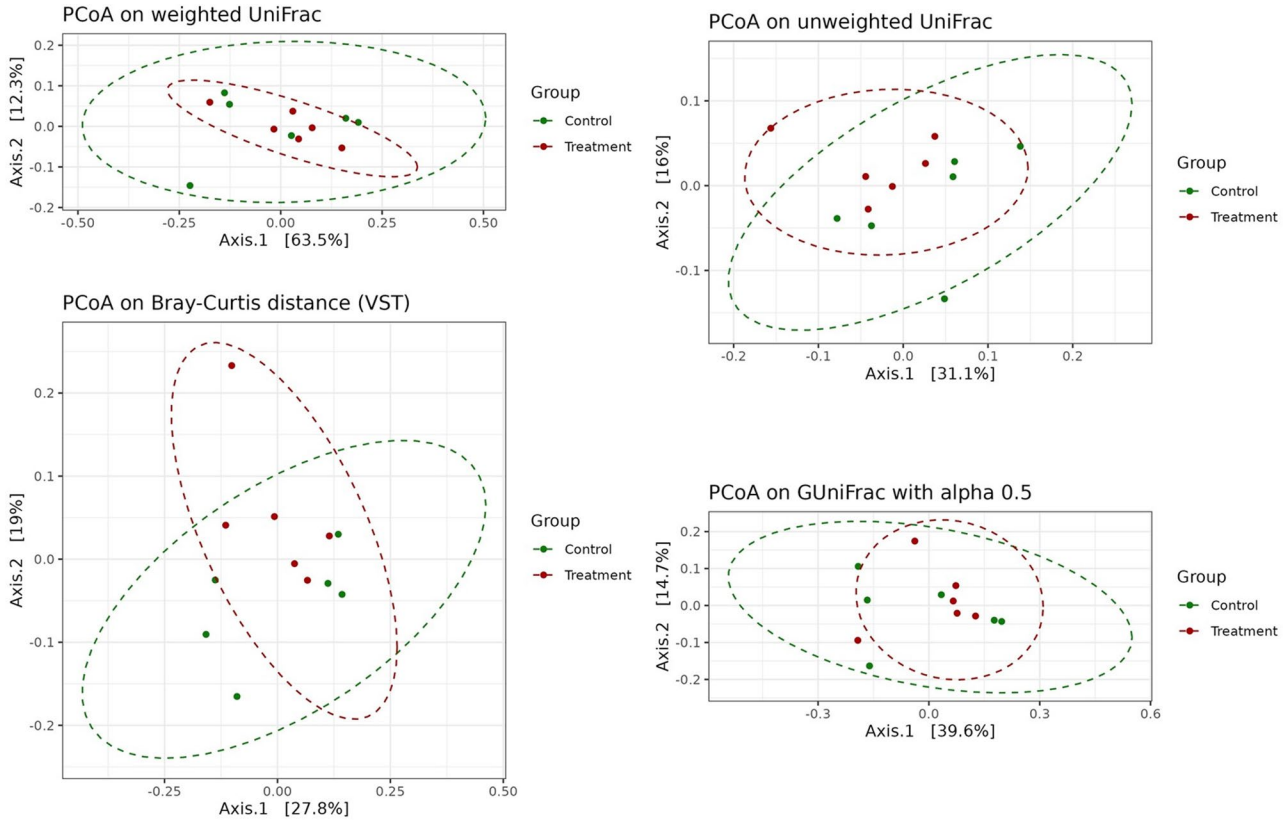

Fig. 5 Beta-Diversity Plots. Beta-diversity analysis using two-dimensional Principal Coordinates Analysis (PCoA) on weighted and unweighted UniFrac distances, Non-Metric Multidimensional Scaling (NMDS) based on Bray–Curtis dissimilarity, and GUniFrac distances, comparing gut microbiota composition between control and kratom-treated groups

difference between the control and kratom treatment groups. Although there was variability in the doses administered across these studies, the results exhibited a consistent trend. The typical human dose of kratom can vary, but it is generally reported to range from 2 to 6 g of kratom powder, either dissolved in fluid or consumed with food, depending on the individual and the intended purpose [28, 29]. The dose of kratom administered to the animals in this study was 300 mg/kg BW, corresponding to approximately 2.142 mg of mitragynine, as determined by the HPLC analysis in Supplementary Data. This dosage was selected based on prior studies that utilized aqueous kratom extracts at doses of 100, 300, and 500 mg/kg BW [22]. Specifically, it was reported that a dose of 300 mg/kg BW of kratom aqueous extract significantly attenuated ethanol withdrawal-induced behaviors, such as rearing, displacement, and head weaving. The doses used in studies reporting positive biological activities of kratom extract vary depending on the specific pharmacological effect being investigated and the model employed. However, a direct comparison across different studies may not always be straightforward due to differences in experimental design, species, and route of administration. To better translate our findings to human relevance, future studies could consider comparing the pharmacokinetics and biological effects of kratom at different doses, including those closer to human consumption levels.

We observed effects of kratom on the histopathology of the rats' intestinal mucosa, with only one of five samples in the kratom treatment group exhibiting focal autolytic changes. In Sprague Dawley rats, histological examination of the heart and lungs following kratom decoction treatment showed no alterations, while changes were noted in the kidney, liver, and brain tissues, without any reported mortality [27]. Thus, the administration of kratom may lead to toxicity in certain organs, including the kidney, liver, and potentially the intestine. Although no significant alterations in hematological parameters or lipid profile were observed in this study, the potential for acute toxicity associated with kratom administration should be considered and further investigated to enable a more comprehensive evaluation of its short-term safety profile. Additionally, liver toxicity should be further investigated through histopathological analysis to gain a deeper understanding of the potential hepatotoxic effects of kratom with prolonged exposure. Incorporating these additional analyses will provide a more comprehensive interpretation of kratom's safety profile.

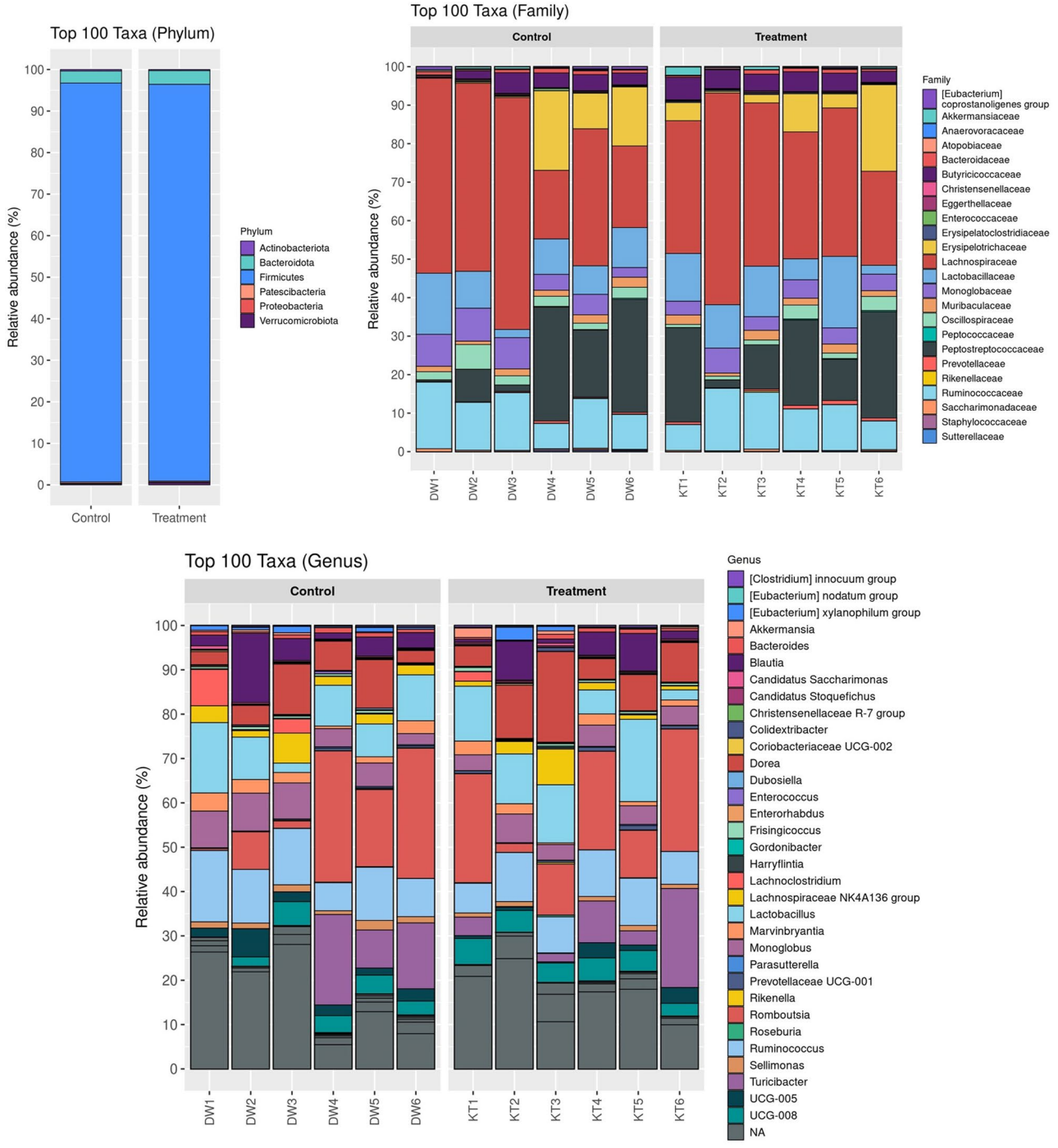

Fig. 6 Taxonomic Profiles of Bacterial Communities. Taxonomic profiles of bacterial communities illustrated at the phylum, family, and genus levels for the control (DW: distilled water) and kratom extract (KT) groups

The water-soluble kratom extract has been shown to modify gut microbiota in fecal batch cultures using 16S rRNA gene sequencing [13]. If kratom affects the gut microbiome in rats, it could be related to gut-brain axis mechanisms, either directly or indirectly, as several microbes are involved in neurotransmitter production. For example, *Lactobacillus* and *Bifidobacterium* can produce γ-aminobutyric acid (GABA), while *Bifidobacterium infantis* has been shown to increase blood plasma tryptophan levels, influencing central serotonin transmission. Species such as *Escherichia*, *Bacillus*, and *Saccharomyces* can produce noradrenaline; *Streptococcus*, *Candida*, *Escherichia*, and *Enterococcus* species can produce serotonin; and various bacteria are capable of

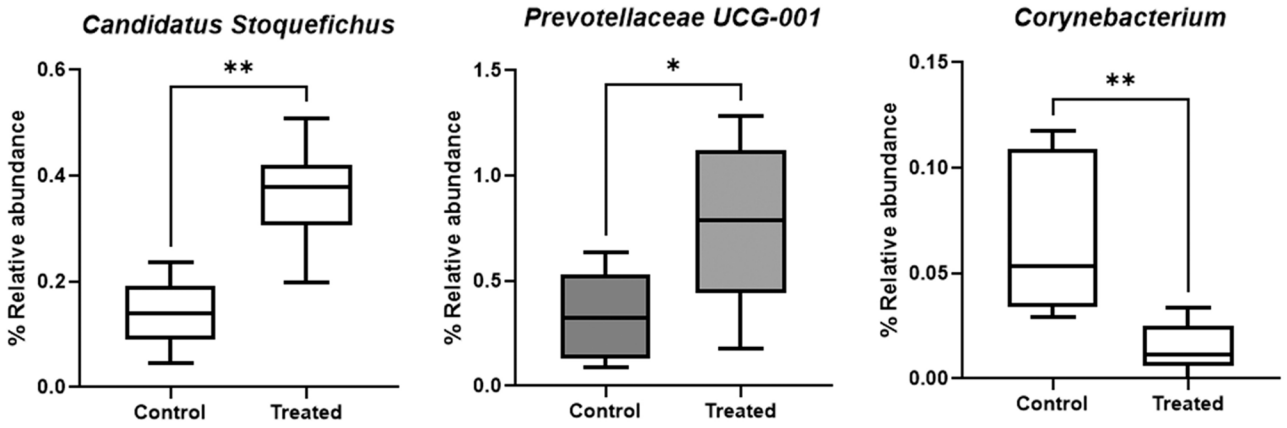

Fig. 7 Alterations in Gut Microbiota Composition. Changes in gut microbiota composition at the genus level following kratom treatment. Statistical significance is indicated with * $p < 0.05$ and ** $p < 0.01$

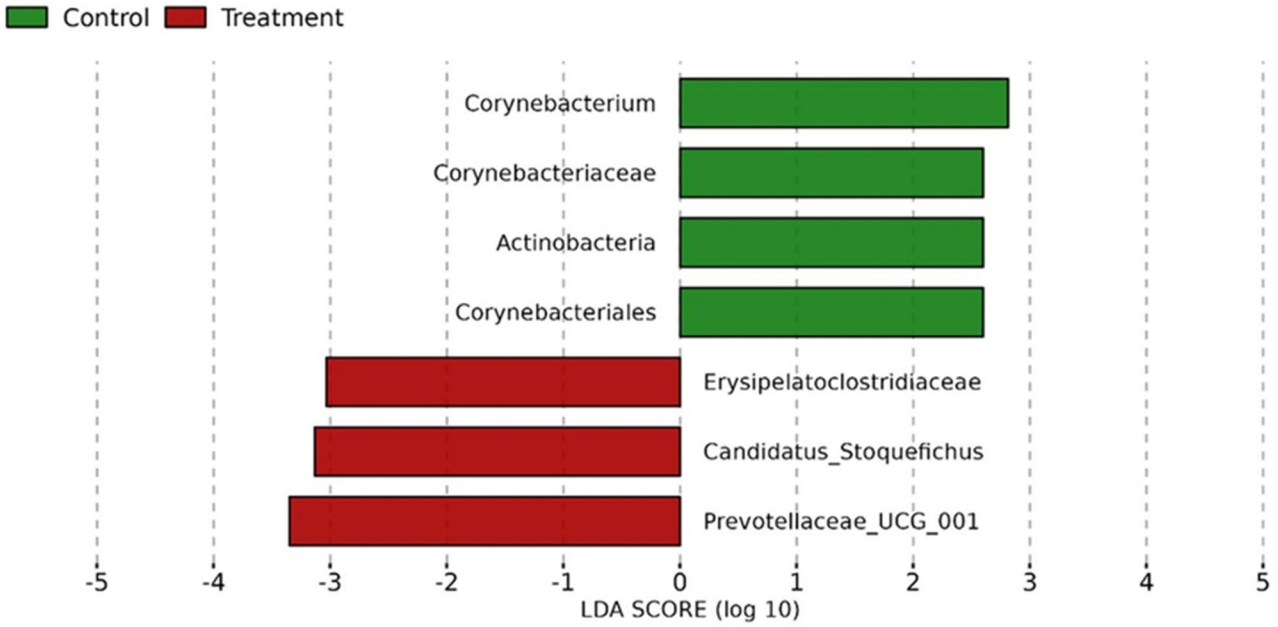

Fig. 8 LEfSe Analysis Bar Plot. Linear Discriminant Analysis (LDA) Effect Size (LEfSe) bar plot representing significant taxa in control (green bars) and kratom treatment (red bars) groups. The length of each bar represents the log10 transformed LDA score

synthesizing dopamine, with *Lactobacillus* also producing acetylcholine [30]. Alterations in neurotransmitter levels have been linked to several neurological and neurodegenerative disorders, including Alzheimer's disease (AD), autism spectrum disorders (ASD), depression, and Parkinson's disease (PD) [31].

In this study, the administration of kratom extract influenced the composition of certain gut microbes in rats. At the family level, the relative abundance of *Corynebacteriaceae* in the treatment group was significantly decreased, while the abundance of *Erysipelatoclostridiaceae* increased significantly compared to the control group. At the genus level, kratom extract treatment significantly increased the abundance of *Candidatus Stoquefichus* and *Prevotellaceae UCG-001*, while reducing the abundance of *Corynebacterium* relative to the control group. A high relative abundance of *Corynebacterium* has been associated with colorectal invasive cancers, suggesting a potential role for this genus in cancer progression [32]. Additionally, *Corynebacterium* has been identified as a marker in the mucosa of Pirc rats, which have a heterozygous germline mutation in *Apc* that leads to spontaneous colon tumor development [33]. These findings imply that kratom extract could have therapeutic potential as an anti-tumor agent; however, further research is needed to explore the mechanisms underlying its anti-cancer effects. Conversely, low levels of *Corynebacterium* may be linked to a depression-like

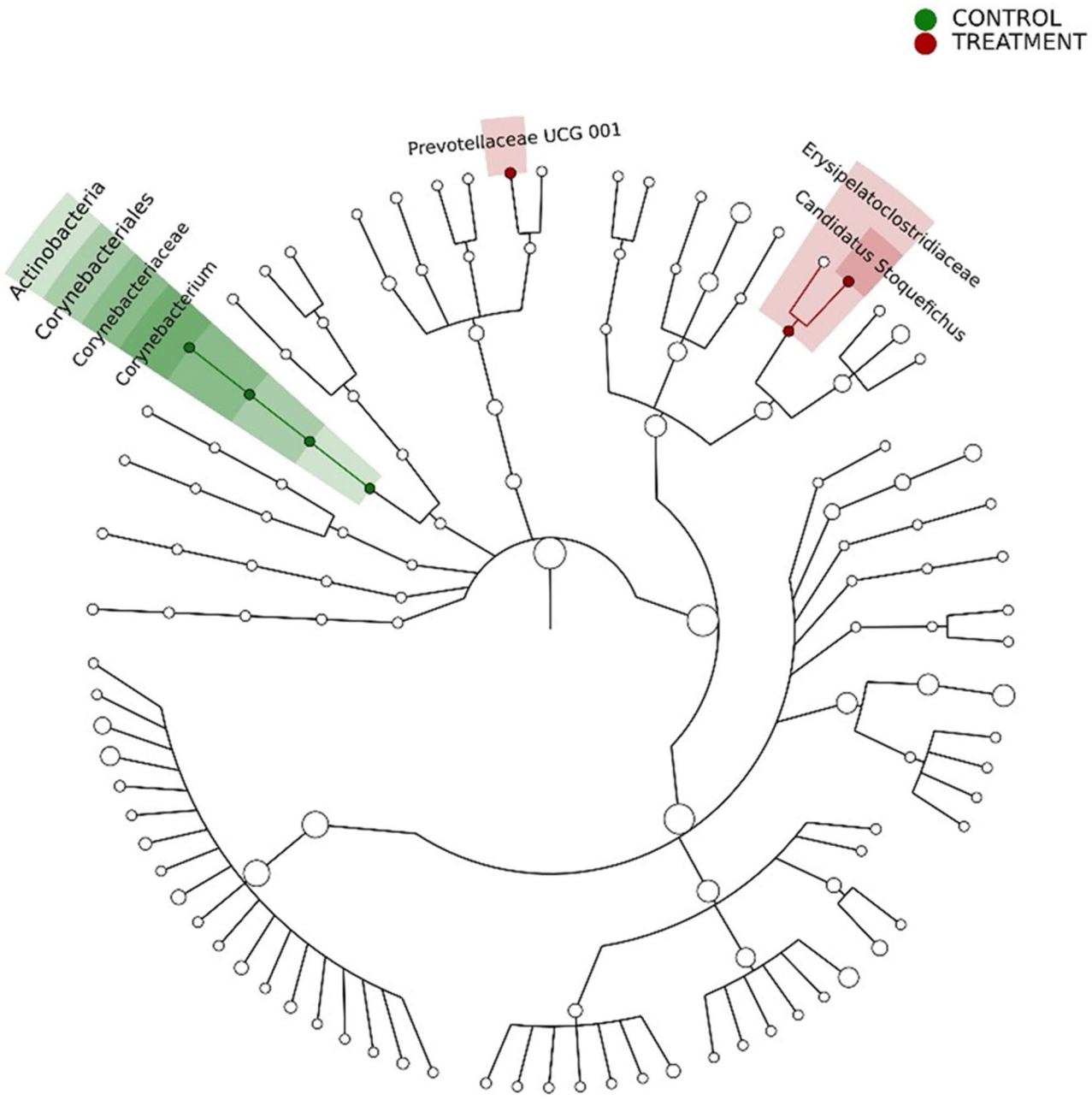

Fig. 9 Cladogram Plot. Cladogram illustrating the hierarchical relationships and significant differences in microbial taxa between groups. Dot colors and shading indicate significantly higher abundance of taxa in each group, with significant phyla shown as central dots and significant genera in the outer circle. The names of significant phyla are indicated in the outermost circle

phenotype in rodents [34, 35]. Therefore, the therapeutic use of kratom should be rigorously evaluated in both animal and clinical studies to mitigate any adverse effects associated with long-term use.

Candidatus Stoquefichus, *Anaerobiospirillum*, *Rikenellaceae RC9* gut group, *Prevotellaceae Ga6A1* group, and *Negativibacillus* are emerging bacterial groups that may induce or worsen colitis [36]. In this study, treatment with kratom extract resulted in focal autolytic changes in the colonic mucosa of one out of five rats. Given the observed increase in *Candidatus Stoquefichus* abundance following kratom treatment, we hypothesize that this bacterium may be linked to changes in the colonic mucosal architecture. However, further investigation is needed to confirm the effects of kratom on colonic mucosa architecture, including studies with larger sample sizes, higher concentrations of kratom extract, or extended treatment durations.

LEfSe and cladogram analyses indicated that the core gut microbiota in the control group included bacteria from the classes *Actinobacteria*, *Corynebacteriales*, *Corynebacteriaceae*, and the genus *Corynebacterium*. In contrast, the treatment group showed a high prevalence of *Erysipelatoclostridiaceae*, *Candidatus_Stoquefichus*, and *Prevotellaceae_UCG_001*. *Erysipelatoclostridiaceae*, recognized as butyrate-producing bacteria, has been linked to various health conditions. For instance, patients with AD or mild cognitive impairment (MCI) exhibited increased levels of *Erysipelatoclostridiaceae* compared to healthy controls ($p < 0.05$) [37]. Similarly, an increase in *Erysipelatoclostridiaceae* has been noted in non-alcoholic fatty liver disease (NAFLD) through 16S NGS analysis [38]. However, LEfSe analysis highlighted differences in gut microbiota composition between major depressive disorder (MDD) and healthy controls, with the latter group showing higher levels of several beneficial gut bacteria, including *Firmicutes* and *Erysipelatoclostridiaceae* [39]. Given that *Erysipelatoclostridiaceae* was prevalent in the kratom treatment group and is associated with diseases such as AD, MCI, and NAFLD, further investigation into kratom's effects and its correlation with the pathogenesis of these conditions is warranted in both long-term animal and clinical studies. Furthermore, both acute and chronic administrations of kratom, which is known to contain the major psychoactive constituent mitragynine, have been associated with cognitive and emotional impairments in several animal studies [40–45]. Alterations in gut microbiota following kratom treatment may be one of the key factors contributing to these impairments.

The abundance of *Prevotellaceae* in fecal samples has been associated with various diseases, including PD, MDD, and stroke. Notably, *Prevotellaceae* may serve as a biomarker for PD, as studies indicate that its abundance in the feces of PD patients is reduced by 77.6% compared to healthy controls [46]. Research on fecal samples from individuals with and without depression has enhanced the understanding of the relationship between gut microbiota and depression [47]. Specifically, *Prevotellaceae* levels are lower in MDD patients compared to healthy individuals. Nevertheless, the abundance of *Prevotellaceae* is linked to stroke severity, though the underlying mechanisms remain unclear [48]. Given that kratom has been shown to increase the abundance of *Prevotellaceae*, a beneficial microorganism, it may offer therapeutic advantages for conditions such as PD or depression. However, further research in both animal models and clinical settings is necessary to fully explore the therapeutic potential of kratom.

Conclusion

This study demonstrated that kratom aqueous extract alters the bacterial composition in the gut of rats. Notably, the abundance of beneficial bacteria, such as *Prevotellaceae*, which may have protective or therapeutic effects for conditions like Parkinson's disease and depression, was increased. However, kratom also increased the abundance of certain bacteria associated with potential negative outcomes. For instance, *Candidatus Stoquefichus* might affect the architecture of the colonic mucosa, and *Erysipelatoclostridiaceae* has been linked to the pathogenesis of Alzheimer's disease, mild cognitive impairment, and non-alcoholic fatty liver disease. These findings highlight the complex effects of kratom on gut microbiota, suggesting that while some changes may be beneficial, others could be detrimental. Given these mixed effects, further research is warranted to explore the impacts of long-term and high-dose kratom use on gut microbiota and its potential implications for overall health.

Abbreviations

AD	Alzheimer's disease
ASD	Autism spectrum disorders
HDL	High-density lipoprotein
LDL	Low-density lipoprotein
LEfSe	Linear discriminant analysis efect size
MCI	Mild cognitive impairment
MDD	Major depressive disorder
NAFLD	Non-alcoholic fatty liver disease
NMDS	Non-metric multidimensional scaling
PCoA	Principal coordinate analysis
PD	Parkinson's disease
RBC	Red blood cell count
RDW	Red blood cell distribution width
WBC	White blood cell count

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12906-025-04836-8.

Supplementary Material 1

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Not applicable.

Author contributions

Thongsepee N and Sornchuer P: Investigation, Sangpairoj K, Martviset P and Chantree P: Assisted Investigation, Thongsepee N, Amonyingcharoen S, Chamod P and Himakhun W: Formal analysis, Thongsepee N and Sornchuer P: Writing– original draft, Sornchuer P: Conceptualization, Writing - review & editing, Funding acquisition, Supervision. All authors reviewed the manuscript.

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Data availability

Sequencing raw data of the gut microbiota in this study was submitted to the NCBI sequence read archive database (BioProject ID: PRJNA1166840). The data can be accessed at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1166840.

Declarations

Ethics approval
All procedures and experiments involving animals were approved by "Thammasat University Animal Care and Use Committee" under Protocol No. 018/2022.

Consent to participate
Not applicable.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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