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SIGNATURES: Roxy Pelteret & Kiara Beilinsohn

A Randomized Controlled Trial of the Effects of Fermented and Green Rooibos on Cognition and Mood in South African Adults at Risk for Cardiovascular Disease

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Abstract

Aspalathus linearis (rooibos) is a widely available natural plant, historically used in traditional indigenous South African medicines. Studies have shown that the biomedical properties of rooibos reduce oxidative stress (OS) and inflammation associated with cardiovascular (CVD) and Alzheimer's disease (AD) and may have neuroprotective effects. However, to our knowledge, no studies have explored rooibos' potential therapeutic or protective effects on cognition and mood in human samples. Thus, this study implemented a randomised placebo-controlled trial to investigate the effects of 12-weeks Fermented Rooibos (FR) and Green Rooibos (GR) consumption on cognition and mood in a sample of South African adults at risk for CVD. Participants *(N* = 235) were randomly assigned to consume FR, GR, or placebo capsules. Pre and post-intervention measures assessed critical cognitive domains, including attention, working and episodic memory, executive functioning, and mental generativity/fluency. Additionally, this study evaluated emotional regulation via heart rate variability (HRV) and self-reported symptoms of anxiety and depression. Results indicated no significant effect of FR or GR on cognition and mood ($p > 0.05$). Given the literature's indication that rooibos modulates OS and inflammation implicated in adverse cognitive and affective outcomes, this research emphasises the need for further investigation.

Keywords: Aspalathus linearis, rooibos, cognition, mood, oxidative stress, cardiovascular disease, Alzheimer’s disease

Aspalathus linearis (rooibos) is an indigenous South African fynbos, most often cultivated to produce "rooibos tea" (Joubert & de Beer, 2011; Marnewick et al., 2011; van Wyk, 2008; Villaño et al., 2010). For centuries rooibos has been used in traditional and indigenous South African medicines (Mahomoodally, 2013; van Wyk, 2008). Numerous studies report that the biomedical properties of rooibos reduce oxidative stress (OS) and inflammation associated with degenerative, neuropsychological, and cardiovascular diseases (CVD) (Marnewick et al., 2011; Pyrzanowska et al., 2019; Salim, 2017). These non-communicable diseases disproportionately affect low and middle-income countries (LAMIC) such as South Africa (RSA) (Beaglehole & Bonita, 2008; Kobayashi et al., 2018; WHO, 2015). Given the consistent link between brain and heart health, rooibos may have a potential therapeutic or protective effect on the neuropathology of CVD and Alzheimer's Disease (AD), with implications for cognition and mood (Bouayed et al., 2009; Maydych, 2019; Sindhu et al., 2022; Toledo et al., 2019).

Biochemical and Medicinal Properties of Rooibos

Rooibos is naturally caffeine-free and rich in minerals. It contains rare polyphenolic compounds, namely, the C-glucoside dihydrochalcones, Nothofagin (Not) and Aspalathin (Asp), which are unique to rooibos (Mahomoodally, 2013; Villaño et al., 2010). Rooibos also contains flavones (luteolin, orientin, isovitexin, vitexin) and flavonols (quercetin and rutin) but does not contain catechins and is low in tannins (Bramati et al., 2003; Marnewick et al., 2011; Pyrzanowska et al., 2019). *Aspalathus linearis* is either left unfermented as green rooibos (GR) or fermented (fermented rooibos (FR)) to produce infusions and extracts (Villaño et al., 2010). FR contains lower levels of Not and Asp and a significantly lower total antioxidant capacity (TAC) than GR due to fermentation-induced phenolic oxidation (Joubert & de Beer, 2011; Joubert et al., 2004; Villaño et al., 2010).

Evidence from animal and human studies illustrates the cardioprotective (Dludla et al., 2014; Marnewick et al., 2011), hepatoprotective (Marnewick et al., 2003; Ulicná et al., 2003), anti-hypertensive (Ajuwon et al., 2015), anti-mutagenic (Marnewick et al., 2009), and anti-diabetic (Kawano et al., 2009; Mazibuko et al., 2015; Muller et al., 2012) medical effects of properties of rooibos. Rooibos may also modulate the immune system, adrenal steroidogenesis, and lipid metabolism (Smith & Swart, 2018).

Anti-inflammatory and Antioxidative Effects

Most literature suggests a linkage between the medicinal properties of rooibos and its antioxidative and anti-inflammatory mechanisms, which protect against damage associated with OS and inflammation (Crichton et al., 2013; Marnewick et al., 2011; Pyrzanowska et al.,

2019). Acute inflammation is a healthy immune response to tissue damage, stress, and infection, which helps maintain homeostasis in the human body (Gronert, 2010). However, unresolved systematic inflammation can cause tissue and neuronal damage and death and increase reactive oxygen species (ROS) production (Hiles et al., 2015; Walker et al., 2019). At low levels, ROS regulates important cell activities related to growth and adaptation; at high levels, it leads to OS (Nita & Grzybowski, 2016; Uttara et al., 2009). OS refers to an imbalance between ROS production and antioxidant defences wherein structures, proteins, and DNA within the body's cells are damaged by a build-up of atomically unstable ROS molecules (Perkins et al., 1999; Toledo et al., 2019). Studies have shown that rooibos inhibits the activity of enzymes involved in ROS production by stimulating the expression of catalase (CAT) and superoxide dismutase (SOD), catalysts for antioxidant activities (Hong et al., 2014; Waisundara & Hoon, 2015).

OS and inflammation are involved in the pathology of CVD and its risk factors (Dludla et al., 2014; Marnewick et al., 2011); diabetes (Kealy et al., 2020); metabolic syndrome (Pyrzanowska et al., 2019); atherosclerosis (Roberts & Sindhu, 2009); hypertension (Kaliora & Dedoussis, 2007); and chronic liver disease (Ajuwon et al., 2015). Studies in healthy human subjects and animals report that rooibos reduces the age-related accumulation of lipid peroxidation (Ajuwon et al., 2015; Joubert & de Beer, 2011; Mahomoodally, 2013). Marnewick and colleagues (2011) findings demonstrate similar beneficial effects in adults at risk for CVD. This study reported that 6-weeks FR consumption (6 cups per day) improved serum lipid profiles and redox statuses by increasing glutathione (GSH) levels, GSH: GSSG ratios, and reducing lipid peroxidation, indicators of OS (Marnewick et al., 2011). Furthermore, reductions in ROS levels and AGE-induced ROS production due to Asp contained in rooibos and correlations between FR consumption and lowered total cholesterol, LDL cholesterol, and triacylglycerols have been reported (Betteridge, 2000; Marnewick et al., 2011; Perkins et al., 1999; Smith & Swart, 2018).

Very few studies have directly examined rooibos' anti-inflammatory capacity. However, there is evidence that its consumption suppresses vascular inflammation in animals (Ajuwon et al., 2015), and in human subjects, reduces high-sensitive C-reactive proteins (hs-CRPs), which are inflammatory markers (Ajuwon et al., 2015; Joubert & de Beer, 2011).

Oxidative Stress and Inflammatory Processes: Cognitive and Affective Outcomes

Given the overlap in risk factors for CVD and AD, a recent interest in the neuroprotective effects of dietary antioxidants such as rooibos has emerged (Sindhu et al., 2022; Smith & Swart, 2018; Toledo et al., 2019). CVD and its risk factors are linked to

cognitive impairments, increased psychological stress, and symptoms of anxiety and depression (Hajjar et al., 2018; Holt et al., 2013; Smith & Swart, 2018; Toledo et al., 2019). Moreover, research indicates that systematic inflammation and neuroinflammation may, directly and indirectly, affect brain function via changes in cardiovascular function (Salim, 2017; Tangestani Fard & Stough, 2019).

OS and inflammation are widely observed in the neuropathology of neurodegenerative disorders such as AD, brain injuries, ischemic strokes and age-related cognitive decline (Akinrinmade et al., 2017; Hajjar et al., 2018; Tangestani Fard & Stough, 2019). In particular, age-related decline in executive functioning in healthy humans and animals is correlated with decreased GSH levels and increased lipid peroxidation, linked to OS and oxidative damage (Dröge & Schipper, 2007; Pesce et al., 2018; Pyrzanowska et al., 2019). Studies have reported that FR tea infusions led to significant long-term spatial memory improvements in healthy rats (Pyrzanowska et al., 2019) and have provided preliminary evidence for its neuroprotective effects in rats with I/R-induced injury (Akinrinmade et al., 2017).

Many of the biomarkers underlying cognitive decline, depression, anxiety and psychological stress, such as the insulin receptor signalling pathway, stress-induced HPA-axis alterations, and high levels of circulating cortisol; overlap with the pathology of CVD and AD (Dröge & Schipper, 2007; Smith & Swart, 2018; Strawbridge et al., 2017). Negative attentional biases, psychological stress, symptoms of anxiety, depression, and ADHD are all associated with defective antioxidant defences and are suggested to have a bi-directional relationship with increased OS and inflammation (Anand et al., 2017; Holt et al., 2013; Maydych, 2019; Smith & Swart, 2018; Zainal & Newman, 2021).

Research suggests that higher levels of OS and pro-inflammatory peripheral cytokines disrupt the antioxidant-oxidant balance in the brain (Salim, 2017; Sindhu et al., 2022; Toledo et al., 2019). This disruption and resultant neuroinflammation may alter neuronal functioning and promote neurodegeneration, cognitive decline, and adverse affective outcomes in healthy human subjects (Huang et al., 2019; Hulbert et al., 2007; Schiavone et al., 2013).

Increases in striatal dopamine, related to emotional regulation and pleasure responses, and elevations in taurine levels, a neuroprotective β-amino acid which may modulate chronic stress, have been reported following FR treatment in animal models (Pyrzanowska et al., 2019; Schaffer & Kim, 2018; Wu & Prentice, 2010). More recently, López and colleagues (2022) found that GR had a more significant anti-anxiolytic effect than diazepam in zebrafish larvae. This study suggested that GR reduced anxiety by rescuing GABA receptor signalling

and suppressing monoamine oxidase A (MAO-A), an enzyme implicated in the deamination of important mood-related neurotransmitters, namely, serotonin, dopamine, and norepinephrine (López et al., 2022).

Thus, rooibos consumption may influence cognitive and affective outcomes through its modulation of OS and inflammation, glucocorticoid biosynthesis and cortisol levels, inhibition of MAO-A, and stimulation of striatial dopamine (Kealy et al., 2020; López et al., 2022; Pyrzanowska et al., 2019; Strawbridge et al., 2017; Waisundara & Hoon, 2015).

Overview

While substantial literature showcases the beneficial affective and cognitive effects of common tea (*Camellia sinensis*) in humans (Mancini et al., 2017), no clinical trials to date have investigated whether or not rooibos produces similar effects. This research is necessary for establishing measurable effects that can potentially be harnessed for therapeutic purposes. Rooibos is widely available, accessible, and has historically been used in traditional and indigenous medicines in RSA, which faces high rates of CVD and AD and a significant treatment gap relating to neurological and mental disorders (Beaglehole & Bonita, 2008; Jack et al., 2014; Kobayashi et al., 2018; WHO, 2015). If proven beneficial, rooibos may present a cost-effective and implementable strategy for increasing dietary intake of natural antioxidants to support cognitive and emotional health (Marnewick et al., 2011; Sindhu et al., 2022; Strawbridge et al., 2017; Toledo et al., 2019). Findings from this study will contribute to the literature on the neuropsychological benefits of rooibos and knowledge of indigenous and traditional South African medicines, which are relied on by many for the management of physical and mental health (Mahomoodally, 2013).

Rationale, Aims, and Hypotheses

This study aimed to investigate the potential effects of 12-weeks consumption of FR and GR on cognition and mood in a sample of South African adults at risk for CVD. It hypothesised that 12-weeks consumption of concentrated FR and/or GR capsules, equivalent to 6 cups worth of daily intake, would improve cognitive functioning and mood in South African adults at risk for CVD (See Table 1 for all hypotheses).

Table 1

Hypotheses Under Investigation

Cognition	Mood
H0.1: There is no difference in cognition pre/post intervention across the three treatment groups.	H0.2: There is no difference in mood pre/post intervention across the three treatment groups.
H1.1: FR and GR supplementation both improve cognitive performance more than the placebo.	H1.2: FR and GR supplementation both improve mood more than the placebo.
H1.2 GR supplementation improves cognitive performance more than FR supplementation	H2.2: GR supplementation improves mood more than FR supplementation

Note. FR = Fermented Rooibos; GR = Green Rooibos

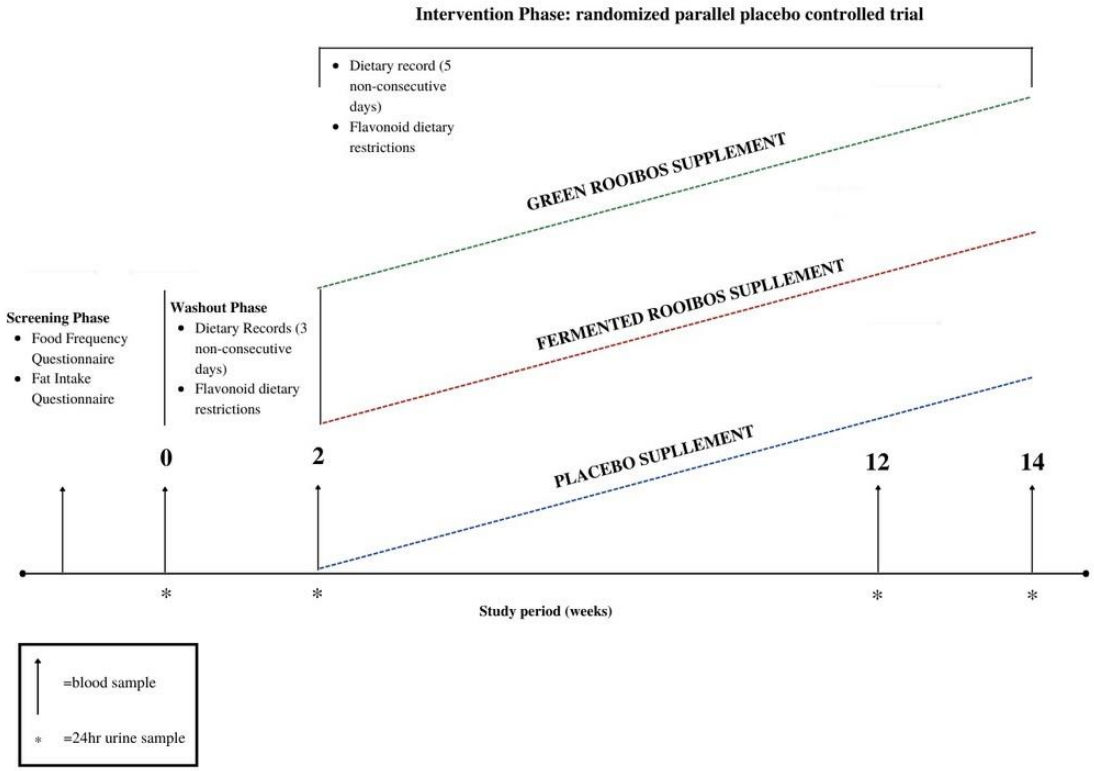
Method

Study Design and Setting

This quantitative study utilised data obtained by the Cape Peninsula University of Technology (CPUT) in the study titled "Rooibos and Heart Health: a metabolomics approach", which investigated the effects of FR and GR on serum levels of OS and inflammation associated with CVD and AD, in South African adults at risk for CVD. This 14-week intervention trial implemented a randomised, double-blind, placebo-controlled, parallel- groups design (see Figure 1). The trial site was the Applied Microbial and Health Biotechnology Institute, CPUT, Bellville, RSA.

Figure 1

Study Design Overview



Note: Measurements and data collection took place at week 2 and 14

Participants

Study Population. Participants included males and females between 30 and 80 years old from the Municipality of Cape Town who were at risk for CVD. This age group and demographic controlled for age-related changes in cognition and, per previous literature, is a clinically relevant sample for the detection of rooibos' potential therapeutic effects (Marnewick et al., 2011; WHO, 2015).

Sample Size. N = 300 participants were recruited, as per results from a 90% power analysis at a significance level of 5%. Due to COVID-19 quarantines, high levels of attrition, and participant factors such as fatigue and difficulty comprehending instructions, the final sample consisted of N = 235 participants.

Recruitment and Screening. Participants were recruited via advertisement (see Appendix A) using convenience sampling, a non-probability method. Registered nurses screened n = 720 individuals for eligibility using health measurements and questionnaires (see Appendix B). N = 300 participants were selected to participate from the pool of participants (n= 450) who met the inclusion and exclusion criteria

Inclusion Criteria. This study included male and female South African adults from Cape Town between the ages of 30 – 80 years with any two or more modifiable risk factors (not requiring any chronic medication) for CVD (see Table 2).

Table 2

Inclusion Parameters

Modifiable Risk Factor for CVD	Inclusion Parameters
Hyperlipidaemia (mmol/L)	Raised Cholesterol (>5.5 to 7.5) Triglycerides (>1.7 to <2.5)
Hypertension (mm Hg)	Existing (≤ 140/90) Prehypertension (120-139/80-90)
Overweight/obese (kg/m2 BMI) Physically Inactive	Body Mass Index (> 25 to 38)
Atherogenic diet (Fat Intake)	High in saturated fat and cholesterol.

Note. Risk Factors as outlined by the American Heart Association (AHA, 2002)

Exclusion Criteria. Table 3 captures participant exclusion criteria. Excluded individuals further included those with values above outlined cut-off points during screening.

Intervention Allocation. The sample was stratified, and treatment groups were matched according to age, sex and CVD risk to minimize potential confounding effects. Participants were then randomly assigned to one of three parallel treatment groups using double-blinding.

Reimbursement. Enrolled participants were reimbursed R300 for time and transport costs for each session.

Table 3

Exclusion Criteria

Criteria	Grounds for Exclusion
Medical conditions	Renal disorders Hepatic disorders Endocrine disorders Gastrointestinal disorders
Undesirable alcohol consumption	>2drinks per day
Unusual dietary habits	e.g. Vegetarian or Vegan diets
Medication/ supplements	Any chronic oral medication Antioxidant supplements Aspirin Any other drug with established antioxidant properties
Pregnancy	Actual/ intended pregnancy Lactating women

Materials and Measures

Capsules. Participants received capsules containing either standardised FR, standardised GR (extracts manufactured by Rooibos Ltd, Clanwilliam, RSA and packaged by an FDA-approved facility) or microcrystalline maltodextrin (placebo). They were required to take three capsules per day, with each meal, for 12 weeks. Supplement dosage was deemed safe based on a prior high-performance liquid chromatography analysis and a previous 6-week clinical trial where participants consumed 6 cups of FR tea daily, with no deleterious effects (Marnewick et al., 2011).

Demographic Variables. A questionnaire recorded participants' gender, age, language, race, and highest education (see Appendix C).

Pre/Post-Intervention Measures. This study utilised the following subset of measures, administered at weeks two and fourteen of the parent study. These measures took 60 minutes to complete.

Cognitive Tests. All cognitive measures were recorded and scored using standardised instructions, as outlined in the battery manuals (see Appendices D to H).

Rey Auditory Verbal Learning Test (RAVLT) (Rey, 1964). The RAVLT was used to assess participants' episodic memory. The administration involved immediate recall and, following a 20-minute interval, delayed recall and recognition of a list of words (see Appendix D). This study utilised delayed recall and recognition scores. The RAVLT has good internal consistency and test-retest reliability (Cronbach's $\alpha = 0.8$) and is used internationally and locally to assess neuropsychological performance (Biedermann et al., 2018; Blumenau & Broom, 2011; Magalhaes et al., 2012; Pliskin et al., 2020).

Digit Span Forwards (DSF)/Backwards (DSB). The Digit Span is a subtest of the Wechsler Adult Intelligence Scale (Wechsler, 1955) and Wechsler Memory Scales (Wechsler, 1945). DSF and DSB assessed attention and working memory, respectively (see Appendix E). In these tests, examiners read participants a sequence of numbers which participants attempted to repeat back in order (DSF) and reverse order (DSB). DSF and DSB have sufficient test-retest reliabilities of Cronbach's $\alpha = 0.89$ and Cronbach's $\alpha = 0.8$, respectively (de Paula et al., 2016; Waters & Caplan, 2003). Working memory and attentional deficits are commonly assessed in international and local contexts using these tests (GrÉGoire & Van Der Linden, 1997; Peltzer & Phaswana-Mafuya, 2012).

Delis-Kaplan Executive Function System Trail Making Test (D-KEFS -TMT) (Delis et al., 2001). This test assessed executive functioning via set shifting and psychomotor speed (see Appendix F). It included a series of 5 timed conditions, each beginning with a short practice trial. This study utilised scores from Condition 4: Number-Letter Switching, which measures cognitive flexibility, a core aspect of executive functioning, via set-shifting. Participants' Trail Switch completion time (TST) and error scores (TSE) were recorded and scaled according to age (see Table G1 and G2 for scaling). International and local studies have used the D-KEFS -TMT and condition four in isolation as they display moderate to good internal consistencies and test-retest reliabilities (Joska et al., 2010; Rosin & Levett, 1989; Swanson, 2005; Pa et al., 2010).

Controlled Oral Word Association test (COWAT) (Benton et al., 1983). The COWAT measured participants' verbal fluency/mental generativity (see Appendix H). This test required participants to list as many words, beginning with a given letter of the alphabet or corresponding to a given semantic category, as they could in 60 seconds. Total scores were calculated by summing correct responses. The COWAT has relevance in diverse populations, including in RSA, and has a high internal consistency and test-retest reliability (Cronbach's $\alpha = 0.83$) (Peltzer & Phaswana-Mafuya, 2012; Ross et al., 2007; Ruff et al., 1996).

Mood Assessments. Heart rate variability (HRV) and self-report questionnaires of depression and anxiety measured participants' emotional states.

Beck Anxiety Inventory- Short Form(BAI-SF) (Beck & Steer, 1993). The BAI measured self-reported anxiety symptoms over the month past (see Appendix I). Responses were in the form of a 4-point Likert Scale ranging from 0 to 3. The 21-item scores were summed to obtain total scores (for score classifications, see Appendix J). The BAI is widely used in diverse populations, including in RSA and is considered valid and reliable, with a high internal consistency (Cronbach's $\alpha = 0.90$ to 0.94) and acceptable test-retest reliability ($r = 0.75$) (Bantjes et al., 2016; Julian, 2011; Kagee et al., 2015; Muntingh et al., 2011).

Beck Depression Inventory-II (BDI-II) (Beck et al., 1996). The BDI-II assessed self-reported symptoms of depression over two weeks past (see Appendix K). Responses ranged from 0 to 3 in the form of a 4-point Likert Scale. Total score calculations involved summing the 21-item scores (see Appendix L for score classifications). The BDI-II has been internationally and locally validated, yielding a good internal consistency (Cronbach's $\alpha = 0.9$) and test-retest reliability ($r = 0.73$ to 0.96) in clinical settings (Makhubela & Mashegoane, 2015; Roberts et al., 1991; Smarr, 2003; Wang & Gorenstein, 2013).

Heart rate variability (HRV). HRV is defined as the fluctuation of the length of heartbeat intervals, reflecting the heart's response to potential challenges (Appelhans & Luecken, 2006; Thayer et al., 2012). HRV was measured using a non-invasive electrocardiographic method (via the Polar H10 portable 1-channel ECG monitor). HRV is an accepted biomarker index of emotional regulation and stress resilience, with low HRV indicative of poorer regulation and resilience (Kim et al., 2018). HRV has been used in a variety of populations (healthy and at-risk), and the Polar H10 has yielded an adequate (0.50–0.75) to good (0.75–0.90) relative reliability in South African samples (Speer et al., 2020).

Procedure

Data collection took place between September 2020 and April 2022. Following the screening phase, selected participants were required to visit the trial site on three occasions. Individuals underwent COVID-19 screening and tests upon entry into the facility.

Visit 1: Screening, Enrolment and Information Session. At visit 1, participants (in groups of 15) signed a sample and study consent form (see Appendix M), were given information about the study and its requirements, and were encouraged to ask/voice any questions/concerns. Each participant was allocated a study code upon enrolment.

2-week Dietary Stabilization Period. For two weeks before baseline assessments, the study requested participants to follow their regular diet with a few dietary restrictions (see

Appendix N for the list of restricted foods and beverages provided). Dietary restrictions included limiting daily consumption of foods/beverages with a high flavonoid content or total antioxidant capacity to 1unit per item to prevent potential confounding effects. Participants also needed to detail their daily meals for three non-consecutive days.

Visit 2: Baseline Data Collection and Allocation. After the 2-week washout period, participants returned for baseline data collection and supplied completed COVID-19 forms and dietary records. Health measurements (larger study's details provided in Appendix O) took place first. After being offered a snack, researchers measured participants' HRV and cognitive and mood tests/questionnaires were completed in a private room. At the end of the session, lasting approximately 2.5 hours, participants were given their R300 reimbursement and issued their capsules.

12-week Intervention Period. During this period, participants were required to take their capsules, complete dietary records, and follow the same dietary restrictions as the 2-week washout phase.

Visit 3: Follow-up. At the follow-up visit, participants provided the same forms and records as visit 2, with the addition of a completed food frequency questionnaire determining their total daily flavonoid intake (see Appendix P). The procedure and measurements of visit two were replicated during this session.

Ethical Considerations

Ethical clearance was granted by CPUT and confirmed by the University of Cape Town (see Appendix Q).

Informed Consent, Anonymity, and Confidentiality. Participants signed a document giving informed consent (see Appendix M). This document detailed the voluntary nature of participation and that participants were free to withdraw from the study at any time without consequences. The aims, procedure, possible risks, and incentives of the study were outlined, and participants were given the contact details of the study leader and doctor for any questions/concerns. Participants who misunderstood or declined to provide informed consent were excluded from participating. Anonymity and confidentiality were maintained as there were no direct participant identifiers in the study, and data was stored in a secure facility.

Risks. This study did not pose any considerable risks. If participants had an adverse reaction to the supplementation, they contacted the registered study doctor. During data collection visits, participants took breaks if they were fatigued and were referred for further neuropsychological tests or to counselling facilities if they exhibited significant cognitive

impairments or mood disturbances. The study implemented and adhered to COVID-19 health and safety regulations.

Benefits. Benefits to participation included undergoing advanced cardiovascular screening and being alerted of any detected abnormalities. Moreover, participants were involved in making a valuable contribution to science.

Debriefing. The study informed participants that, following publishing, jargon-free summaries would be made available (see Appendix R).

Data analysis

Quantitative data, including HRV, cognitive and mood data, was collated and imported into an excel database. Blind data analysis took place using the statistical-based software "R".

Variables. The independent variable for cognition and mood was the intervention received (placebo, GR, or FR). Highest education level (HLE), sex, age, and risk for cardiovascular disease, via Body Max Index (BMI) and cholesterol, were used as controls (see Table 4 for dependent variables)

Table 4

Dependent Variables for Mood and Cognition

Cognition		Mood	
Measure	Outcome	Measure	Outcome
D-KEFS Trial Making Test: Number-Letter Switching	Cognitive Flexibility	Beck Anxiety Inventory- Short Form	Anxiety
Rey Auditory Verbal Learning Test: Delayed Recall and Recognition	Episodic memory	Beck Depression Inventory-II	Depression
Controlled Oral Word Association	Mental Generativity/ Verbal Fluency	Heart Rate Variability	Emotional Regulation & Stress resilience
Digit Span Forwards	Attention		

Digit Span Backwards	Working memory

Note. D-KEFS= Delis Kaplan Executive Function System Trail Making Test

Data Management. Outliers, values above Q3 + 3xIQR or below Q1 – 3xIQR, were removed from specific cognitive or mood measure data (Metcalf & Casey, 2016). When appropriate, missing values were replaced using mean value substitution for completeness (Kang, 2013). For the categorical control variable of highest education level (HLE), each level of education was assigned a numerical value (Primary School = 1; Highschool = 2; Undergraduate Degree = 3, and Postgraduate degree = 4). Similarly, males and females were assigned to 1 and 2, respectively.

Descriptive and Inferential Analysis. Descriptive statistics were generated and presented in table format. These statistics and general trends and distributions across groups were visualized and inspected. ANOVAs assumptions of homogeneity of variance and normality were checked using Levine's Tests and Shapiro Wilks Tests and QQ plots of residuals, respectively. One-way ANOVAs were conducted to investigate baseline differences between the three-intervention group's cognitive and mood scores. 3 x 2 Factorial ANOVAs with covariates were then utilized to test the related cognitive and mood hypothesis (see Table 1) (Borm et al., 2007). Session time (baseline or follow-up) and group (intervention type) were used as the main effects, and an interaction effect of session and group was included. The variable of "participant ID" was treated as a random effect to account for the idiosyncratic variation due to individual differences (Correll et al., 2021).

The nature and directionality of differences for significant ANOVA findings were assessed using Post-Hoc analyses. These analyses were done using Independent Samples T-tests or Contrasts with Pairwise Comparisons and Tukey Adjustments, controlling for Type 1 Errors. Effect sizes for significant findings were explored (Noble, 2009).

Results

Final Sample Size

This study's final total sample size consisted of $N = 235$ unique participants, split into three overlapping samples. The cognitive sample included $n = 160$ participants, while the three mood samples, BAI, BDI, and HRV, consisted of $n = 200$, $n = 201$, and $n = 132$, respectively.

Cognitive data

Three outliers were identified and removed from both RAVLT recognition and delayed recall, while one was removed from DSF. Refer to Table 5 for sample characteristics.

Table 5

Cognitive Sample Clinical and Demographic Characteristics

Variable	GR	FR	Control	Total Sample
Number of subjects	52	50	58	160
Age, Mean ± SD (Range)	45±10.21 (30-65)	49±10.23 (30-71)	46±10.6 (30-71)	47 ±10.42(30-73)
Sex, *n (%)*				
Females	39(75)	39 (80.77)	40(68.97)	118 (73.75)
Males	13(25)	11 (22)	18(31.03)	42 (26.25)
*HLE, Mean ±SD	2.4±0.5	2.2±0.74	2.4±0.7	2.34±0.7
BMI, Mean (range)	31.33 (18.76 – 59.31)	30.27 (18.02-47.80)	31.89 (15.89-51.93)	31.20 (15.89 – 59.31)
Cholesterol, Mean (range)	5.46(4.16-7.72)	5.49(4.11-7.67)	5.38(3.88-7.73)	5.5(4.11-7.73)
High, n	0	3	3	6
Low, n	1	2	3	6

Note. HLE = Highest education level,

BMI= Body Max Index.

*HLE 2= High School

Descriptive statistics. Table 6 depicts descriptive statistics of pre/post-intervention cognitive scores across the three treatment groups

Inferential Statistics. ANOVA's assumption of homogeneity of variance was found to be upheld for all cognitive measures: D-KEFS TST (p = .67) and Errors (p = .26); RAVLT Recognition (p = .34) and Delayed Recall (p = . 37); COWAT (p = .42); DSF (p = .51); and DSB (p = .73) while normality assumptions were violated for all measures (p < .05).

Table 6

Descriptive Statistics for Pre/Post Intervention Cognitive Scores Across Treatment Groups

Measure		GR				FR				Control			
		N	Mean ±SD	Min	Max	*N*	Mean ±SD	Min	Max	*N*	Mean ±SD	Min	Max
D-KEFS TMT													
Switch time	Baseline	52	7.67±4.06	1	14	50	6.84±4.37	1	14	58	7.12±4.01	1	17
	Follow-up		8.62±3.9	1	14		7.4±4.29	1	14		8.95±3.32	1	12
Switch Errors	Baseline	52	10.15±2.07	6	12	50	8.8±3.42	1	12	58	8.95±3.32	1	14
	Follow-up		9.63±2.6	1	12		9.74±2.13	3	12		9.5±2.81	1	12
RAVLT													
Recognition	Baseline	52	12.33±2.44	7	15	50	11.9±2.96	3	15	55	12.67±2.74	6	23
	Follow-up		12.58±2.14	4	15		12.06±2.68	5	15		11.53±3.84	0	15
Delayed Recall	Baseline	52	9.33±3.55	3	15	50	8.4±3.42	2	15	55	8.64±3.37	1	15
	Follow-up		8.21±3.53	0	14		7.1±3.47	1	15		7.62±3.43	2	15
COWAT	Baseline	52	62.44±15.96	35	113	50	58.22±19.08	19	116	58	60.39±15.21	20	102
	Follow-up		57.7±16.54	33	109		55.88±18.76	19	92		57.82±16.47	26	99
DSF	Baseline	52	8.42±2.15	5	15	50	8.48±1.98	4	14	57	8.88±2.07	4	15
	Follow-up		8.83±2.28	6	16		8.96±2.43	4	15		9.23±2	6	15
DSB	Baseline	52	5.31±2.14	2	12	50	4.58±2.03	2	10	58	4.91±1.82	2	11
	Follow-up		4.85±2.03	2	11		4.76±1.71	2	10		4.91±2	2	11

Note. D-KEFS TMT = Delis Kaplan Executive Function System Trail Making Test: Switch time and errors= Number-Letter Switching completion time and errors respectively; RAVLT= Ray Auditory Verbal Learning Test; COWAT= Controlled Oral Word Association; DSF= Digit Span Forwards; DSB= Digit Span Backwards.

This assumption remained violated after data transformation, including scaling and log transformations. However, research indicates that ANOVAs are robust and may be viably implemented under violated normality assumptions (Keselman et al., 2001).

Baseline Differences in Cognition. One-way ANOVA results showed no significant group differences in baseline scores for D-KEFS TST ($F(2, 157) = 0.54$, $p = .585$); RAVLT Recognition ($F(1, 158) = 0.44$, $p = .507$) and Delayed Recall ($F(1, 158) = 1.86$, $p = .175$); COWAT ($F(2, 157) = 0.81$, $p = .447$) DSF ($F(1, 158) = 0.02$, $p = .886$); and DSB ($F(2, 157) = 1.71$, $p = .185$). However, baseline D-KEFS TSE scores differed significantly ($F(2, 157) = 3.19$, $p = .044$, $\eta p2 = 0.04$) between intervention groups. These findings indicate that cognitive functioning was equivalent across groups at baseline, except for D-KEFS TSE scores, measuring cognitive flexibility, for which the GR group had the highest ($M = 10.15$, $SD = 2.07$) and the FR group had the lowest ($M = 8.8$, $SD = 3.42$) baseline scores, respectively. The difference between baseline error scores suggests that potentially significant changes in cognitive flexibility may be due to these differences as opposed to the applied intervention.

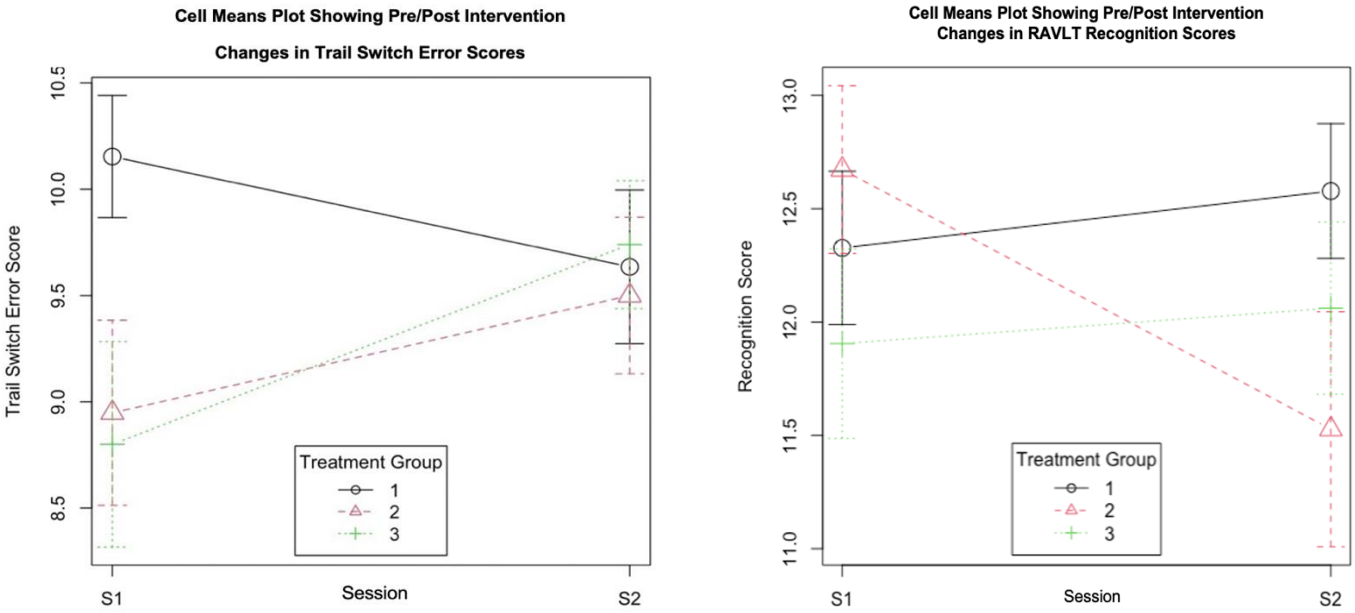
The Effects of Rooibos on Cognition. Factorial ANOVA results indicated that the main effect of the intervention had no significant impact on cognitive scores (see Appendix S for full Type III ANOVA outputs). On the other hand, the main effect of session time (baseline or follow-up) was found to be significant for D-KEFS TST ($F(2, 157) = 8$, $p = .005$, $\eta p2 = 0.04$); RAVLT delayed recall ($F(2, 154) = 36.74$, $p = .00$, $\eta p2 = .07$); COWAT ($F(1, 157) = 16.91$, $p = .00$, $\eta p2 = 0.08$); and DSF scores ($F(1, 156) = 8.62$, $p = .004$, $\eta p2 = 0.02$) with effect sizes ranging from small to medium.

Post-Hoc Independent T-Tests results revealed a significant overall increase in D-KEFS TST scores ($t(157) = -2.89$, $p = .005$) and a decrease in COWAT ($t(157) = 4.02$, $p = .00$) and RAVLT delayed recall ($t(154) = 6.06$, $p < .001$) scores from baseline to follow-up. memory, and verbal fluency/mental generativity at the follow-up. These results indicate that, overall, participants had poorer cognitive flexibility, verbal fluency/ mental generativity, and episodic memory at the follow-up. On the other hand, a significant improvement in follow-up DSF scores, measuring attention, was found ($t(156) = -2.94$, $p = .003$) (see Table T1 for significant pre/post intervention descriptive statistics).

Factorial ANOVA outputs further demonstrated a significant interaction effect of session time and intervention on D-KEFS TSE ($F(2, 157) = 3.13$, $p = .046$, $\eta p2 = 0.03$) and RAVLT recognition scores ($F(2, 154) = 3.43$, $p =. 035$, $\eta p2 = 0.03$). Cell Means Plots (Figure 2) depicted these interactions as disordinal.

Figure 2

Cell Means Plots for Group X Session D-KEFS Trail Switch Error and RAVLT Recognition



Note. I Bars are at 95%

Treatment group 1 = Green Rooibos; Treatment group 2 = Control; Treatment group 3= Fermented Rooibos

Figure 2 indicates that the intervention type had different effects on D-KEFS TSE scores depending on the time of measurement. Separate simple effects analysis indicated that the FR group's error scores increased significantly ($t(157) = -2.166$, $p = .032$), and thus, cognitive flexibility declined from baseline ($M = 8.8$, $SD = 3.42$) to follow-up ($M = 9.74$, $SD = 2.13$). Moreover, findings showed that the control group's episodic memory ($t(154) = 2.75$, $p = .007$) declined from baseline ($M = 12.67$, $SD = 2.74$) to follow-up ($M = 11.53$, $SD = 3.84$). These findings must be interpreted with caution given the disordinal nature of the interaction and because of one-way ANOVA results demonstrating the GR group lower baseline scores.

The Effects of Covariates on Cognition. Factorial ANOVAs showed that HLE had a significant effect on all cognitive scores: D-KEFS TST ($F(3, 150) = 9.49$, $p = .00$, $\eta p2 = 0.13$) and error scores ($F(3, 150) = 3.61$, $p = .014$, $\eta p2 = 0.05$); RAVLT recognition ($F(3, 147) = 3.57$, $p = .016$, $\eta p2 = 0.05$) and delayed recall ($F(3, 147) = 7.91$, $p = .00$, $\eta p2 = 0.12$); COWAT ($F(3, 150) = 16.29$, $p = .00$, $\eta p2 = 0.22$); DSF ($F(3, 149) = 13.96$, $p = .00$, $\eta p2 = 0.18$) and DSB ($F(3, 150) = 7.38$, $p = .00$, $\eta p2 = 0.12$). The effect sizes for HLE ranged from small to medium for all measures, apart from the large effect size found for COWAT and DSF. Mean investigation revealed that higher education levels were associated with better

performance for all cognitive domains measured (see Table T2 for HLE Descriptive Statistics).

The covariate of sex was found to have a significant effect on D-KEFS TST (F(1, 150) = 36.04, p = .006, ηp2 = 0.04); RAVLT recognition (F(1, 147) = 4.24, p = .04, ηp2 = 0.02) and delayed recall (F(1, 147)= 12.52, p = .001, ηp2 = 0.07); and DSB (F(1, 150) = 3.03, p = .083, ηp2 = 0.02). Small effect sizes were identified for all cognitive measures except for RAVLT Delayed recall, which was medium (ηp2 = 0.07). One-sided independent T-tests demonstrated that females scored higher than males on these cognitive tasks: D-KEFS TST (t(142.5)= -3.35, p = .001); RAVLT recognition (t(177.26) = -2.9, p = 0.002) and delayed recall (t(212.45) = -5.42, p = .00); and DSB (t(182.85) = -2.32, p = .012) (see Table T3 for descriptive statistics of cognitive scores for males and females).

Summary of Cognitive Findings. Given that no significant effect of the intervention was observed in the factorial ANOVAs, it can be inferred that changes in cognition between baseline and follow-up assessments were due to causes unrelated to the intervention type. This implies that neither GR nor FR showed a statistically significant ability to improve cognitive functioning.

Mood data

Three outliers were identified and removed for BDI, and eight outliers for BAI were removed. Appendix U captures the demographic and clinical characteristics of each mood sub-sample, BAI (n = 192), BDI (n = 198), and HRV (n = 132), across the three intervention groups.

Descriptive Statistics. Table 7 summarises the descriptive statistics for pre/post-intervention mood scores across the intervention groups.

Inferential Statistics. As with the cognitive data, ANOVA's assumption of normality was violated for all mood measures: BAI (p = .00), BDI (p = .00) and HRV (p = .007) and remained violated following data manipulation. However, homogeneity of variance was found to be upheld: BAI (p = .430), BDI (p = .392), and HRV (p = .968). Given their allowance for violated assumptions of normality, ANOVAs were then conducted (Keselman et al., 2001).

Baseline Differences in Mood. No significant group differences were found in baseline scores for BAI (F(1, 190) = 0.84, p = 0.36, ηp2 = 0.04); BDI (F(1, 195) = 0.05, p = .824, ηp2 = 0.00); and HRV (F(1, 129) = 0.21, p = .812, ηp2 = 0.00).

The Effects of Rooibos on Mood. Results demonstrated that differences between the three intervention groups' pre/post intervention BAI, BDI, and HRV scores were non-

significant. This indicated that the type of intervention, FR and/or GR, had no significant impact on mood scores. See Appendix V for the full Type III ANOVA outputs for all mood measures. The interaction effect of session and intervention was identified as non-significant for all mood measures. However, a significant main effect of session for BAI (F(1, 157) = 10.72, p = .001, ηp2 = 0.04) and BDI (F(1, 163) = 6.09, p = .0015, ηp2 = 0.03) was found, with small effect sizes.

Post-Hoc simple effects analyses for the effect of session time revealed that BAI (t(157) = 3.28 p = .001) and BDI (t(163) = 2.47, p = .015) scores decreased significantly from baseline (BAI: M = 6.81, SD = 7.02, BDI: M = 6.82, SD = 6.45) to follow-up (BAI: M = 5.28, SD = 6.26, BDI: M = 5.65, SD = 6.36), suggesting lower levels of anxiety and depression, respectively.

The Effects of Covariates on Mood. The covariate sex had a significant effect on BAI (F(1,150) = 5.27, p = .023, ηp2 = 0.02) and BDI scores (F(1,156) = 3.14, p = .078, ηp2 = 0.01) with one-sided Independent Samples T-tests demonstrating that females had higher levels of anxiety (t(190) = -3.37, p = .00) and depression (t (192) = -2.76, p = .003) than males. Moreover, age had a significant effect on all mood measures, including BAI (F(1,150) = 13.62, p = .00, ηp2 = 0.05), BDI (F(1,156) = 11.35, p = .01, ηp2 = 0.05) and HRV (F(1, 100) = 4.11, p = .045, ηp2 = 0.027) scores (see Appendix W for means and SD across sex and age groups).

The cholesterol covariate only had a significant effect on BDI (F(1,156) = 5.08, p = .026, ηp2 = 0.02) with individuals with low cholesterol having higher levels of depression (M = 11.82, SD = 12.94) than those with high cholesterol (M = 5.14, SD = 3.78).

Summary of Mood Findings. Like cognition, although significant pre/post-intervention changes were found, results indicate that the intervention type did not affect these changes. Thus, findings suggested that neither GR nor FR had any effect on mood.

Table 7

Descriptive statistics for pre/post intervention mood scores across the three treatment groups

Measure		GR				FR				Control			
		N	Mean ±SD	Min	Max	*N*	Mean ±SD	Min	Max	*N*	Mean ±SD	Min	Max
BAI	Baseline	65	7.97±7.95	0	29	60	6.87±7.46	0	36	67	5.63±5.38	0	21
	Follow-up		5.01±6.05	0	29		5.83±6.79	0	31		5.04±6.03	0	30
BDI	Baseline	65	6.94±5.76	0	30	68	6.69±7.64	0	32	61	6.83±6.03	0	28
	Follow-up		6.42±7.05	0	27		5.94±7.01	0	29		4.69±4.89	0	27
HRV	Baseline	41	53.3±10.9	30	93	39	53.5±10.0	27	72	52	52.3±8.92	30	71
	Follow-up		52.8±9.12	27	71		51.8±9.51	31	71		52.6±10.03	29	71

Note: BAI = Beck's Anxiety Inventory, BDI= Beck's Depression Inventory, HRV= Heart Rate Variability.

Discussion

This study aimed to investigate the effects of 12-weeks consumption of rooibos on cognition and mood in a sample of South African adults at risk for CVD. Literature demonstrates that rooibos has beneficial antioxidative, anti-inflammatory, and neuroprotective properties (Marnewick et al., 2011; Pyrzanowska et al., 2019). Therefore, we analysed if and to what degree FR and GR could improve cognitive performance on neuropsychological tests and reduce symptoms of anxiety and depression. To the best of our knowledge, this study constitutes the first investigation of the effects of rooibos supplementation on neuropsychological functioning in a human sample. Results indicated that neither GR nor FR supplementation caused post-intervention changes in cognition or mood.

In vivo studies on rooibos have shown that its neuroprotective effects are due to its ability to counteract ROS production and reduce levels of proinflammatory cytokines (Hong et al., 2014; Waisundara & Hoon, 2015). Previous literature demonstrates that rooibos can modulate OS and inflammation associated with impaired cognitive functioning by increasing GSH levels and decreasing lipid peroxidation in healthy humans and animals (Hong et al., 2014; Marnewick et al., 2011; Pyrzanowska et al., 2019). Moreover, research indicates that GR has a significantly higher TAC and contains higher Not and Asp levels than FR (Joubert & de Beer, 2011). Given these findings, we suspected that cognition would improve in the rooibos groups and that GR supplementation would result in more significant cognitive improvements. However, our results diverge from the suggestion made by previous studies in that no significant changes in cognition attributable to FR or GR occurred. Rooibos is not a medication, and aspects of our study design and methodology may account for these null findings.

Prior research provides evidence of a potential mechanistic link between rooibos and neuropsychological health via reduced OS and inflammation associated with cognitive impairments (Akinrinmade et al., 2017; Tangestani Fard & Stough, 2019). For instance, many studies report the beneficial effects of rooibos on lipid peroxidation and GSH levels based on pre-clinical models of Alzheimer's disease and neurological injury (Akinrinmade et al., 2017; Darvesh et al., 2010) and in samples of individuals with age-related cognitive decline (Ajuwon et al., 2015; Joubert & de Beer, 2011; Mahomoodally, 2013). Nevertheless, they do not provide direct evidence that rooibos improves cognition *per se*. We are aware of only one study that directly explored cognition in animals, finding that 12 weeks of daily FR administration improved spatial memory in rats (Pyrzanowska et al., 2019). In this regard, a

direct comparison is impracticable because we did not measure spatial memory. Therefore, our null findings may be attributed to this failure to assess spatial memory in conjunction with the measures used to evaluate other cognitive domains.

The cognitive tests used in this study are designed to detect clinically significant cognitive declines (Bauer & Malek-Ahmadi, 2021). Therefore, these tools may not have been sensitive enough to detect subtle changes in cognition in our neurologically healthy sample. The cognitive measures we used are also susceptible to ceiling and floor effects, which may have caused an underestimation of the impact of FR or GR on cognitive performance (Scherr et al., 2016). Although many studies on other herbal beverages, such as green tea, have demonstrated improvements in cognitive functioning using similar test batteries (Cascella et al., 2017; Mancini et al., 2017), they were retrospective or longitudinal in design and often included samples with mild cognitive impairment. We surmise that the potential cognitive benefits of rooibos in humans may only be detectable in response to regular, life-long consumption.

The reported anomalous findings in the cognitive data suggest that some extraneous or confounding factors may have been at play in this study. Across all samples, results indicated that measurement time significantly impacted all cognitive domains apart from working memory. Declines in cognitive performance from baseline to follow-up, except in attention, were observed. These changes were not attributable to FR or GR, nor can they be attributed to age-related cognitive decline, given the relatively short time between assessments. Due to the ongoing COVID-19 pandemic at the time of the trial, we cannot exclude the possibility that some participants may have had COVID-19 over the intervention period, and as recent research suggests, post-COVID syndrome negatively impacts cognition (Evered, 2022; Suárez-González et al., 2021).

While the sample size may not have been large enough to cancel out most potential confounding variables, the finding here that education was associated with better cognitive performance indicates that the tests did capture valid variations in neuropsychological performance. Consistent with evidence suggesting that the number of years of formal education is positively correlated with increased cognitive functioning in adults, this study found that higher education levels were associated with better performance on all cognitive tasks (Lövdén, et al., 2020). More surprisingly, females performed better on almost all cognitive measures, which is unexpected given the notion that there are no sex/gender differences in brain anatomy and cognitive functioning (although see Asperholm et al., 2019; Jäncke, 2018;). Our finding is likely due to the significantly greater proportion of females as

opposed to males in this sub-sample (73% females) and the study's total sample, which suggests that sampling may have been a source of error in the analyses.

This study also assessed the impact of rooibos on mood due to the mechanisms by which it can modulate OS, inflammation, glucocorticoid biosynthesis, and cortisol levels (Kealy et al., 2020; Strawbridge et al., 2017; Waisundara & Hoon, 2015). Although there is little existing data to suggest that rooibos is directly beneficial in treating depression, many of the biomarkers underlying CVD and AD overlap with those of depression, anxiety and psychological stress (Strawbridge et al., 2017). These biomarkers include the insulin receptor signalling pathway, stress-induced HPA-axis alterations, and high levels of circulating cortisol (Dröge & Schipper, 2007; Smith & Swart, 2018; Strawbridge et al., 2017). Thus, we hypothesised that mood would improve in the rooibos groups and potentially, given that GR has a higher TAC than FR, GR would result in more significant mood improvements. However, no beneficial effects of rooibos on depression, anxiety, or HRV emerged.

Regarding depression levels, these findings are somewhat surprising given that studies have reported increased striatal dopamine, associated with emotional regulation, motivation, and reward systems, in response to long-term FR treatment in rats (Pyrzanowska et al., 2019). We suspect that in humans, rooibos-related improvements in depression may similarly require long-term administration. Additionally, study designs likely necessitate direct observations of antioxidant and neuroinflammatory serum level changes that can be linked to shifts in depression. These direct investigations are required given that adverse affective outcomes are associated with defective antioxidant defences, which have a bi-directional relationship with increased OS and inflammation (Anand et al., 2017; Holt et al., 2013; Maydych, 2019; Smith & Swart, 2018; Zainal & Newman, 2021).

This study's null finding regarding anxiety and HRV is inconsistent with several pre-existing studies which suggest that rooibos is anxiolytic. Animal studies have shown that rooibos prevents lipid and protein peroxidation in stressed rats by inhibiting enzyme activities involved in ROS production and stimulating CAT and SOD expression (Hong et al., 2014; Waisundara & Hoon, 2015). There are also reports that rooibos elevates taurine levels, a neuroprotective β-amino acid which may modulate chronic stress, in rats (Schaffer & Kim, 2018; Wu & Prentice, 2010). Similarly, rooibos has been shown to rescue GABA receptor signalling and suppress MAO-A in animals, with anxiolytic effects, in zebra fish larvae (López et al., 2022).

We cannot discount the fact that skewed baseline measures of depression and anxiety may explain our null findings. Research has consistently shown that CVD and its risk factors,

in addition to cognitive impairments, are implicated in increased psychological stress and symptoms of depression and anxiety (Hajjar et al., 2018; Holt et al., 2013; Smith & Swart, 2018; Toledo et al., 2019). While the current sample was at risk for CVD, this study found only mild and minimal baseline levels of anxiety and depression. Given the retrospective nature of these tests, participants may have struggled to accurately remember their feelings over the previous two or three weeks. Moreover, they could have felt uncomfortable sharing the potentially sensitive information requested by these questionnaires.

Self-report measures like the BDI/BAI are also highly vulnerable to social desirability bias, in which participants often underreport negative emotional states due to the stigma associated with mental health issues (Krumpal, 2013). Cultural and socio-economic factors may have further impacted scores in that cultures diverge in the expression of affective experience through a somatic versus psychological lens (Lee et al., 2017). We did observe some patterns in the mood data consistent with the literature, namely, that females, individuals with low cholesterol, and the youngest cohort (ages 30-39) had the highest self-reported negative affect. However, given our other findings, we suspect that responses to items on these measures may not have accurately reflected participants' true feelings, which would explain the unexpectedly low levels of anxiety and depression amongst the sample population.

To overcome issues related to self-report measures, HRV was incorporated into the design. However, no improvements in HRV were observed between the treatment groups. Moreover, as indexed by higher HRV, better psychological resilience was associated with the oldest age group in this study. This finding is incongruent with evidence suggesting that HRV declines with age (Kumral et al., 2019). Together, these findings from the HRV and mood data sets suggest that the current protocol of rooibos supplementation was ineffective in modulating emotional states, but whether this was due to the protocol itself or confounds related to the sample size and trial compliance remains indeterminate.

Indeed, several unanticipated confounding variables emerged. For example, although FR nor GR improved mood in the sample, results indicated that across all three intervention groups, levels of anxiety and depression decreased from baseline to follow-up. Studies have consistently shown that high and low-stress levels decrease and increase mood, respectively (de Rooij et al., 2010). We suspect that the time of year may have influenced results as baseline assessments were conducted at the end of 2021 during COVID-19 restrictions in RSA, while follow-up assessments took place at the start of 2022 after the holidays and when

COVID-19 restrictions had eased. These unexpected effects compound the issues related to sample size and representativeness.

Limitations and Directions for Future Research

Aside from problems related to sample size and time of year, the current study was limited by certain factors, the first of which pertains to experimental control. Given that the 12-week intervention period took place outside of a controlled laboratory, there may have been multiple extraneous factors contributing to the changes, or lack thereof, observed in post-intervention scores. In particular, this study lacked a comprehensive method for verifying whether participants were adhering to the required procedure of taking three capsules daily. If participants failed to take their capsules in a timely and dedicated manner, results might have been affected, and post-intervention changes in cognition and mood may have been hindered. Thus, future studies may benefit from weekly compliance checks using objective measures such as blood tests.

Literature suggests that the mechanisms by which rooibos may potentially improve mood and cognition involve its reduction of OS and inflammation and modulation of glucocorticoid biosynthesis and cortisol levels (Kealy et al., 2020; Strawbridge et al., 2017; Waisundara & Hoon, 2015). However, this study did not include or analyse data on FR's and GR's direct effects on participants' serum levels of OS and inflammation or cortisol. Data of this nature could provide valuable information about the effects of rooibos and allow for sub-group analysis of changes in mood and cognition for participants with varying degrees of rooibos-related changes in serum levels of OS and inflammation. Thus, future research should consider the combined use of neuropsychological cognitive tests, subjective mood questionnaires, and biomarkers.

The process of data capturing revealed a disparity between participants' item responses on the BAI and BDI, measuring their levels of anxiety and depression, respectively. Multiple participants indicated traumatic experiences such as abuse and expressed suicidal ideation. However, high scores for these items existed in isolation, with total scores being very low. The quantitative nature of these self-report measures may thus have been a limitation of this study. Assessment tools that do not rely on self-reported mood are preferable, given their ability to overcome limitations and biases associated with introspection. Future studies may benefit from using a mixed methods approach combining quantitative data from health measurements and mood questionnaires and qualitative data obtained via clinical interviews. This approach may provide a more comprehensive clinical picture of participants' emotional states and, thus, enable more accurate results about the

effects of rooibos on mood. Concerning the cognitive measures utilised in this study, many are designed to detect mild to severe cognitive impairments, which were not a distinctive feature in the current sample (Bauer & Malek-Ahmadi, 2021). Therefore, future studies conducted in similar sample populations should employ more sensitive measures such as reaction times to detect potential changes.

Although potentially unavoidable, the R300 reimbursement and food offered by this study may have been a motivating and, thereby, a confounding factor in the study (Meloy et al., 2006). The monetary reward appeared to cause an influx of participants who signed up based on this reimbursement without a due understanding of what participation would involve. Future studies will have greater success with compliance and participant motivation if recruitment methods emphasise what participation will involve prior to communicating reimbursement.

Finally, another limitation of the study linked to sampling pertains to convivence sampling. Although enabling access to more participants, this method resulted in an unequal proportion of males to females (73.75% females) in overall and sub-samples for cognition and mood. This inequality may present a limitation regarding the generalisability of findings, as research indicates a systematic sex difference in self-reported bias (Lines et al., 2021; Hunt et al., 2003). Therefore, we recommend that future studies include a larger and more representative sample to eliminate confounding effects, increase statistical power, and provide more ecologically valid results.

Conclusion

Literature on rooibos' neuroprotective and stress-reducing effects has been steadily growing over the last two decades. However, this research is mainly confined to in vivo and animal models. Therefore, this study sought to address the lack of human studies by investigating the potential effects of 12-weeks consumption of FR and GR on cognition and mood in a sample of South African adults at risk for CVD. As the first placebo-controlled trial of its kind, this study contributes to the limited knowledge base on rooibos' neuroprotective and therapeutic effects on mood and cognition. Despite the finding that rooibos did not result in cognitive or affective improvements in this sample, substantial evidence from animal and in vivo studies has demonstrated its modulation of oxidative stress and inflammation, implicated in cognitive impairments, increased psychological stress, and symptoms of anxiety and depression. Given the biochemical mechanisms by which rooibos may improve cognition and mood, future studies on the acute effects of rooibos in human participants will require carefully controlled experimental designs that utilise sensitive

biophysiological measures that can detect subtle changes in neuropsychological functioning.

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Appendix A

Participant Recruitment Advertisement

ROOIBOS STUDY SEEKS VOLUNTEERS

Male and female volunteers are invited to participate in a **"first of its kind"**, **14 week research study** to investigate the possible heart health benefits of the popular South African herbal tea, ROOIBOS.

Participation requirements (only two required)

- Age between 30 and 80 years
- Increased blood cholesterol and/or sugar levels
- Family history of heart disease
- Increased Body Mass Index (kg/m²)

All potential volunteers should

- Not take any chronic oral medication
- Have no clinically significant abnormalities of the liver, kidneys or blood
- Not be pregnant (or intend to fall pregnant within the next few months)

Benefits

- Learn more about your own health status
- Learn more about Rooibos & contribute to much needed research in the field
- All study materials will be provided to you at no cost
- Questionnaire-based assessment of stress and cognition with feedback

If you are interested in taking part in this study, please get in touch with the study coordinator at the contact details below to receive more information and assess your eligibility for participation.

Email Rooibostrial@cput.ac.za or Rooibostrial@gmail.com
Cellphone/WhatsApp 082 876 1064

Oxidative Stress Research Centre
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Cape Peninsula University of Technology
creating futures

Appendix B

Screening Health Questionnaire

Cape Peninsula University of Technology

Health questionnaire

Participant number:	**Date:**
Date of birth:	**Age:**

	Yes	No	Unsure
1. Do you have high blood pressure?			
2. Do you smoke?			
3. Do you have low blood sugar/diabetes?			
4. Do you have a family history of heart disease?			
5. Do you have elevated cholesterol levels?			
6. Do you drink alcohol?			
If yes, please indicate type of alcohol and frequency:			
6.1 Wine			
6.1.1) Red wine			
6.1.2) White wine			
6.2 Frequency 6.2.1 Not more than two (2) glasses per day			
6.2.3 More than two (2) glasses per day			
6.3 Beer			
6.4 Frequency 6.4.1 Not more than two (2) drinks per day			
6.4.2 More than two (2) drinks per day			
6.5 Spirits			
6.5.1 Brandy			
6.5.2 Whiskey			

6.5.3 Rum			
6.6 Frequency 6.6.1 Not more than two (2) drinks per day			
6.6.2 More than two (2) drinks per day			
	Yes	**No**	**Unsure**
7. Do you use life sustaining medication for conditions such as:			
7.1 Cholesterol?			
7.2 Blood pressure?			
7.3 Diabetes?			
7.4 Other (Please specify)			
8. Are you physically active? (≥30 minutes moderate intensity exercise most days of the week)			
9. Did your body weight remain stable for the past 6 (six) months?			
10. Do you use dietary supplements?			
a. If yes, state frequency			
b. Describe the dietary supplements used			
11. Do you follow any diet specific diet such as:			
a. Vegetarian or vegan diet?			
b. High protein diet?			
c. Healthy eating habits incorporated in diet?			
d. Other (Please specify)			
12. Do you have any kidney problems?			
13. Do you have any liver diseases or problems?			
14. If female, are you pregnant (actual or intended)?			

Appendix C

Demographic Questionnaire

Cape Peninsula University of Technology

STUDY TITLE

Demographic questionnaire

Section A: General

1.1 Participant number			
1.2 Date	D	M	Y

Contact telephone number: _____

Please mark your response with an "X" in the appropriate response box

		Response
1.4 Your gender?	Male	1
	Female	2

1.5 Your age in years? (Please indicate)			
1.6 Your date of birth? (Please indicate)	D	M	Y

1.7 Your first language?	1.7.1 Afrikaans	1
	1.7.2 English	2
	1.7.3 Xhosa	3
	1.7.4 Other	4

1.8 Your second language?	1.8.1 Afrikaans	1
	1.8.2 English	2
	1.8.3 Xhosa	3
	1.8.4 Other	4

		Response
1.9 Your marital status?	1.9.1 Never married	1
	1.9.2 Married	2
	1.9.3 Divorced	3
	1.9.4 Widowed	4

1.10 What is your occupation?	
1.10.1 **Legislator, senior official and manager** *(e.g. CEO, president/vice president, general manager, divisional head, postmaster, superintendent, dean, school principal)*	1
1.10.2 **Professional** *(e.g. engineer, architect, lawyer, biologist, geologist, psychologist, accountant, medical doctor, town planner)*	2
1.10.3 **Associate professional and technician** *(e.g. computer programmer, nurse, physio/occupational therapist, actor, photographer, illustrating artist, product designer, translator, pilot, broker, quality inspector)*	3
1.10.4 **Clerk** *(e.g. bookkeeper, teller, cashier, messengers and office helper, typist, telephone operator, secretary, reception clerk, library clerk)*	4
1.10.5 **Service and sales worker** *(e.g. nurses' aid, hairdresser, guide, housekeeper, childcare, fire-fighter, advertising agent, real estate agent, sales clerk, shop attendant)*	5
1.10.6 **Skilled agricultural and fishery worker** *(e.g. farmer, grower, planter, winemaker, horticultural worker, fisherman/woman)*	6
1.10.7 **Craft and related trades** *e.g. miner, quarry worker, bricklayer, carpenter, plasterer, plumber, electrician, painter, mechanic, locksmith)*	7
1.10.8 **Plant and machine operator** *e.g. truck driver, bus driver, taxi driver, sound and video recorder, textile worker, production machine worker)*	8
1.10.9 **Elementary occupation** *e.g. news vendor, garage attendant, car washer, gardener, farm labourer, garbage collector, sweeper)*	9
1.10.10 **Learner/student**	10

1.11 Your highest level of education?	1.11.1 Secondary school/High school	1
	1.11.2 Matric (St.10/Grade 12)	2
	1.11.3 Certificate	3
	1.11.4 Diploma	4
	1.11.5 Degree	5
	1.11.6 Post-graduate degree (Masters/Doctoral)	6

Section B: Lifestyle

2. Smoking		Response
2.1 Do you smoke?	2.1.1 Never smoked	1
	2.1.2 Former smoker	2
	2.1.3 Current smoker	3
2.2 If you currently smoke, please indicate your selection	2.2.1 Cigarettes	1
	2.2.2 Tobacco/pipe	2
	2.2.3 Cigar	3

3. Physical activity		
3.1 Are you physically active?	3.1.1 Yes	1
	3.1.2 No	2
3.2 If yes, how regularly do you exercise (mark the appropriate section)?		
	3.2.1 1x/week	1
	3.2.2 3x/week	2
	3.2.3 > 3x/week	3
3.3 Would you describe your physical activity level as:		
3.3.1 Moderately active - walking briskly (about 5½ km per hour); hiking; gardening; dancing; golf (walking and carrying clubs); bicycling (less than 16 km per hour); weight training (general light workout)?		1
3.3.2 Vigorously active - running/jogging (8 km per hour); bicycling (more than 16 km per hour); Swimming (freestyle laps); aerobics; walking very fast (7 km per hour); Heavy work, such as chopping wood; Weight lifting (vigorous effort)?		2

Appendix D

Rey Auditory Verbal Learning Test (RAVLT) Test

REY AUDITORY VERBAL LEARNING TEST

Use the word list printed below for most regular visits. You should use the RAVLT Alternate Word List (not included in this booklet) when the participant has already been tested using the word list shown below within the past year. Regardless of which word list you use, you should indicate here the form that was used:

- ◯ Regular word list (printed below)
- ◯ Alternate word list (use separately printed English-language word list)
- ◯ Spanish word list (use separately printed Spanish-language word list)

Trial I: *"The next task may seem a bit difficult in the beginning, but usually it gets easier as we go along. I am going to read for you a long list of words. Once I'm done, I'd like to see how many of the words you can recall. You can repeat the words in any order that you prefer; you don't have to use the same order that I use. Then, I am going to read the same list for you a few more times, to see how many of the words you can eventually learn. Ready?"*

Trial II: *"That was a good beginning. Now I'm going to read the same list again, and again I would like to see how many of the words you can recall, including the words you remembered on the first trial. Again, listen very carefully. Ready?"*

Trials III–V: *"Very good. I'm going to read the list again. Again, listen carefully and try to remember as many words as you can. Ready?"*

	TRIAL I	TRIAL II	TRIAL III	TRIAL IV	TRIAL V
1	Drum	Drum	Drum	Drum	Drum
2	Curtain	Curtain	Curtain	Curtain	Curtain
3	Bell	Bell	Bell	Bell	Bell
4	Coffee	Coffee	Coffee	Coffee	Coffee
5	School	School	School	School	School
6	Parent	Parent	Parent	Parent	Parent
7	Moon	Moon	Moon	Moon	Moon
8	Garden	Garden	Garden	Garden	Garden
9	Hat	Hat	Hat	Hat	Hat
10	Farmer	Farmer	Farmer	Farmer	Farmer
11	Nose	Nose	Nose	Nose	Nose
12	Turkey	Turkey	Turkey	Turkey	Turkey
13	Color	Color	Color	Color	Color
14	House	House	House	House	House
15	River	River	River	River	River

PAGE 2

Missing = "99"

CORRECT: TRIAL I TRIAL II TRIAL III TRIAL IV TRIAL V

ERRORS: TRIAL I TRIAL II TRIAL III TRIAL IV TRIAL V

REY AUDITORY VERBAL LEARNING TEST
(Interference List, Recall Following Interference)

Instructions: After Trial V of the primary word list, say *"Very good. I want you to try to remember as many of those words as possible because I'm going to ask you about them again a little later." Then say, "Now I am going to read for you a different list of words. Once again, when I'm done, I'd like to see how many of the words you can recall. Ready?"* Read the interference list (desk, ranger, etc.) and record responses under Trial VI.

After the subject has recalled as much as possible from the interference list, say, *"Now I'd like to see how many words you can recall from the first list—the one we went through five times. Tell me as many words as you can remember from the first list."* Record responses under Trial VII.

	TRIAL VI	TRIAL VII
1	Desk	Drum
2	Ranger	Curtain
3	Bird	Bell
4	Shoe	Coffee
5	Stove	School
6	Mountain	Parent
7	Glasses	Moon
8	Towel	Garden
9	Cloud	Hat
10	Boat	Farmer
11	Lamb	Nose
12	Gun	Turkey
13	Pencil	Color
14	Church	House
15	Fish	River

Missing = "99"

CORRECT: TRIAL VI TRIAL VII

ERRORS: TRIAL VI TRIAL VII

THE REY COMPLEX FIGURE COPY AND IMMEDIATE RECALL SHOULD BE ADMINISTERED NEXT.

SERIAL #

REY AUDITORY VERBAL LEARNING TEST
(Delayed Recall)

Instructions: Without reading the list again, say: *"Remember the long list of words we went through five times? I'd like you now to tell me as many of the words from that list as you can remember."*

	TRIAL VIII
1	Drum
2	Curtain
3	Bell
4	Coffee
5	School
6	Parent
7	Moon
8	Garden
9	Hat
10	Farmer
11	Nose
12	Turkey
13	Color
14	House
15	River

Missing = "99"

TRIAL VIII — CORRECT: 0 0, 10 1, 2, 3, 4, 5, 6, 7, 8, 90 9

TRIAL VIII — ERRORS: 0 0, 10 1, 20 2, 30 3, 40 4, 50 5, 60 6, 70 7, 80 8, 90 9

REY AUDITORY VERBAL LEARNING TEST
Recognition (Trial IX)

Show the subject the list of words and say, *"Next I would like to see how many of these words you can recognize. Please circle all of the words on this list that you think were part of the original list that we went through five times. Make sure you only circle those words that you are sure you remember."*

TRIAL IX

RECOGNITION HITS: 0 0, 10 1, 2, 3, 4, 5, 6, 7, 8, 90 9

Missing = "99"

RECOGNITION FALSE POSITIVES: 0 0, 10 1, 20 2, 30 3, 4, 5, 6, 7, 8, 90 9

PARTICIPANT CODE:

BELL	HOME	TOWEL	BOAT	GLASSES
WINDOW	FISH	CURTAIN	HOT	STOCKING
HAT	MOON	FLOWER	PARENT	SHOE
BARN	TREE	COLOUR	WATER	TEACHER
RANGER	BALLOON	DESK	FARMER	STOVE
NOSE	BIRD	GUN	ROSE	NEST
WEATHER	MOUNTAIN	CRAYON	CLOUD	CHILDREN
SCHOOL	COFFEE	CHURCH	HOUSE	DRUM
HAND	MOUSE	TURKEY	STRANGER	TOFFEE
PENCIL	RIVER	FOUNTAIN	GARDEN	SHEEP

Appendix E

Digit Span Forwards & Backwards Administration Procedure

Participant Id#:

Date: ☐☐ / ☐☐ / ☐☐☐☐
Month Day Year

DIGIT SPAN TEST - - *FORWARD*

- After saying the instructions administer the digit spans in order.
- Do not repeat a span once read.
- Administer both spans of the same length regardless of how the participant performs.
- Say the digits at a rate of 1 digit about every 1 sec.
- Use a monotonic voice; without inflections at the end
- Discontinue after failure on both trials of any item (e.g., 5a and 5b)

Examiner: *"I am going to say some numbers. Listen carefully, and when I am through say them right after me. For example, if I say 7-1-9, what would you say?"*

- If the participant responds correctly (7-1-9), say: *"That's right,"* and proceed to Item 1.
- If the participant fails the example, say: *"No, you would say 7-1-9. I said 7-1-9, so to say it forwards you would say 7-1-9. Now try these numbers. Remember, you are to say them forwards. 3-4-8."*
- Whether the participant succeeds or fails with the second example (3-4-8), proceed to Item 1. Give no help on this second example or any of the items that follow.

Scoring: Each span is scored '1' (Pass) or '0' (Fail). Only discontinue test when participant has failed both trials of the same span length (e.g., 5a and 5b)

Item	Digit Span	Pass	Fail
1 a.	1 - 7	○ 1	○ 0
b.	6 - 3	○ 1	○ 0
2 a.	5 - 8 - 2	○ 1	○ 0
b.	6 - 9 - 4	○ 1	○ 0
3 a.	6 - 4 - 3 - 9	○ 1	○ 0
b.	7 - 2 - 8 - 6	○ 1	○ 0
4 a.	4 - 2 - 7 - 3 - 1	○ 1	○ 0
b.	7 - 5 - 8 - 3 - 6	○ 1	○ 0
5 a.	6 - 1 - 9 - 4 - 7 - 3	○ 1	○ 0
b.	3 - 9 - 2 - 4 - 8 - 7	○ 1	○ 0
6 a.	5 - 9 - 1 - 7 - 4 - 2 - 8	○ 1	○ 0
b.	4 - 1 - 7 - 9 - 3 - 8 - 6	○ 1	○ 0
7 a.	5 - 8 - 1 - 9 - 2 - 6 - 4 - 7	○ 1	○ 0
b.	3 - 8 - 2 - 9 - 5 - 1 - 7 - 4	○ 1	○ 0
8 a.	2 - 7 - 5 - 8 - 6 - 2 - 5 - 8 - 4	○ 1	○ 0
b.	7 - 1 - 3 - 9 - 4 - 2 - 5 - 6 - 8	○ 1	○ 0

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DIGIT SPAN TEST - - *BACKWARD*

- Administer the digit spans in order.
- Do not repeat a span once read.
- Administer both spans of the same length regardless of how the participant performs.
- Say the digits at a rate of 1 digit about every 1 sec.
- Use a monotonic voice; without inflections at the end

Examiner: *"Now I am going to say some numbers, but this time when I stop I want you say them backwards. For example, if I say 7-1-9, what would you say?"*

- If the participant responds correctly (9-1-7), say: *"That's right,"* and proceed to Item 1.
- If the participant fails the example, say: *"No, you would say 9-1-7. I said 7-1-9, so to say it backwards you would say 9-1-7. Now try these numbers. Remember, you are to say them backwards. 3-4-8."*
- Whether the participant succeeds or fails with the second example (3-4-8), proceed to Item 1. Give no help on this second example or any of the items that follow.
- Discontinue after failure on both trials of any item (e.g., 5a and 5b)

Scoring: Each span is scored '1' (Pass) or '0' (Fail). Only discontinue test when participant has failed both trials of the same span length (e.g., 5a and 5b)

Item	Digit Span	Pass	Fail
1 a.	2 - 4	○ 1	○ 0
b.	5 - 7	○ 1	○ 0
2 a.	6 - 2 - 9	○ 1	○ 0
b.	4 - 1 - 5	○ 1	○ 0
3 a.	3 - 2 - 7 - 9	○ 1	○ 0
b.	4 - 9 - 6 - 8	○ 1	○ 0
4 a.	1 - 5 - 2 - 8 - 6	○ 1	○ 0
b.	6 - 1 - 8 - 4 - 3	○ 1	○ 0
5 a.	5 - 3 - 9 - 4 - 1 - 8	○ 1	○ 0
b.	7 - 2 - 4 - 8 - 5 - 6	○ 1	○ 0
6 a.	8 - 1 - 2 - 9 - 3 - 6 - 5	○ 1	○ 0
b.	4 - 7 - 3 - 9 - 1 - 2 - 8	○ 1	○ 0
7 a.	9 - 4 - 3 - 7 - 6 - 2 - 5 - 8	○ 1	○ 0
b.	7 - 2 - 8 - 1 - 9 - 6 - 5 - 3	○ 1	○ 0

Appendix F

Delis-Kaplan Executive Function System (D-KEFS) Trail Making Test

Trail Making Test

Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Visual Scanning Response Booklet
- Pen (any color)
- Stopwatch

Discontinue

Discontinue after 150 seconds.

Condition 1: Visual Scanning

Place the stimulus booklet in its easel position. Place the Visual Scanning Response Booklet flat on the table and facing the examinee so that he or she can easily write on the practice page. Give the examinee a pen. Point to the practice page and say,

> **Here are some numbers and letters. I want you to find all of the threes on this page. Make a mark like this each time you see a three** (draw a slash through the 3 that is in the upper-left quadrant of the box from the examinee's perspective). **Don't place marks on any of the other numbers or letters, just the threes. Mark the threes as quickly as you can without missing any. Go ahead.**

Correct and explain any errors. After the examinee has completed the practice task, say,

> **Good. Now let's try this one.**

Even if the examinee had difficulty with the practice task, administer the Visual Scanning condition. Open the response booklet to the second and third pages. Place it flat on the table directly in front of the examinee, horizontally at the examinee's midline. Say,

> **Here are more numbers and letters. Like before, I would like you to mark all of the threes on these two pages. Mark the threes as quickly as you can without missing any. Tell me when you are finished. Ready? Begin.**

Start timing. When the examinee has marked all the threes or indicated that he or she has finished, stop timing. Record the total time in seconds on the record form.

If the examinee fails to finish the task by 150 seconds, say,

> **Stop. That's good.**

If the examinee has begun marking a number or letter at the end of the time limit, allow the examinee to complete that response before telling him or her to stop. This response is scored as completed within the time limit.

Do not allow the examinee to mark any numbers or letters after the time limit.

Remove the Visual Scanning Response Booklet.

Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Number Sequencing Response Booklet
- Pen (any color)
- Stopwatch

Discontinue

If the examinee cannot complete the practice task ***after four corrections*** by the examiner, discontinue the practice task and do not administer the scored Number Sequencing condition. Proceed to Condition 3: Letter Sequencing.

Discontinue the scored Number Sequencing condition after 150 seconds.

Note

If the examinee has difficulty holding the response booklet stationary, hold down the top or side edges of the booklet with your fingertips.

Condition 2: Number Sequencing

Leave the stimulus booklet in its easel position. Place the Number Sequencing Response Booklet flat on the table and facing the examinee so that he or she can easily write on the practice page. Point to the practice page and say,

> **Here are some more numbers and letters. This time, I want you to connect *just the numbers*. Begin at number one** (point to the 1) **and draw a line from one to two** (trace this connection with your finger), **two to three** (trace this connection with your finger), **three to four** (trace this connection with your finger), **and so on, in order, until you reach the end** (point to the 5). **Draw the lines as quickly as you can without making mistakes. Go ahead.**

If the examinee makes an incorrect connection, stop him or her immediately after he or she has completed the connection. Write an *X* over the incorrect connection, explain the error, and point to the correct connection. Ask the examinee to proceed from the last correctly connected number. If the examinee completes the practice task with fewer than four errors, administer the Number Sequencing condition. Say,

> **Good. Now let's try this one.**

Discontinue

Discontinue the scored Number Sequencing condition after 150 seconds.

Note

If the examinee has difficulty holding the response booklet stationary, hold down the top or side edges of the booklet with your fingertips.

Condition 2: Number Sequencing *(continued)*

Open the response booklet to the second and third pages. Place it flat on the table directly in front of the examinee, horizontally at the examinee's midline. Say,

> **On this page are more numbers and letters. Do this the same way by connecting *just the numbers*. Begin at number one** (point to the 1) **and draw a line from one to two** (trace this connection with your finger), **two to three** (trace this connection with your finger), **three to four** (trace this connection with your finger), **and so on, in order, until you reach the end** (point to the 16). **Draw the lines as quickly as you can without making mistakes. Ready? Begin.**

Start timing. If the examinee makes an incorrect connection, stop him or her immediately after he or she has completed the connection. Write an *X* over the incorrect connection, state that he or she has made an error, and, ***without explaining the error***, ask him or her to proceed from the last correctly connected number. Keep the stopwatch running while pointing out errors.

When the examinee completes the last connection (reaches number 16), stop timing. Record the total time in seconds on the record form.

If the examinee fails to finish the task by 150 seconds, say,

> **Stop. That's good.**

Allow the examinee to complete any connection in progress at the time limit before telling him or her to stop. This connection is scored as completed within the time limit.

Remove the Number Sequencing Response Booklet.

Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Letter Sequencing Response Booklet
- Pen (any color)
- Stopwatch

Discontinue

If the examinee cannot complete the practice task ***after four corrections*** by the examiner, discontinue the practice task and do not administer the scored Letter Sequencing condition or Condition 4: Number–Letter Switching. Instead, proceed to Condition 5: Motor Speed.

Discontinue the scored Letter Sequencing condition after 150 seconds.

Note

If the examinee has difficulty holding the response booklet stationary, hold down the top or side edges of the booklet with your fingertips.

Condition 3: Letter Sequencing

Leave the stimulus booklet in its easel position. Place the Letter Sequencing Response Booklet flat on the table and facing the examinee so that he or she can easily write on the practice page. Point to the practice page and say,

> **This time, I want you to connect *just the letters*. Begin at the letter *A*** (point to the *A*) **and draw a line from *A* to *B*** (trace this connection with your finger), ***B* to *C*** (trace this connection with your finger), ***C* to *D*** (trace this connection with your finger), **and so on, in order, until you reach the end** (point to the letter *E*). **Draw the lines as quickly as you can without making mistakes. Go ahead.**

If the examinee makes an incorrect connection, stop him or her immediately after he or she has completed the connection. Write an *X* over the incorrect connection, explain the error, and point to the correct connection. Ask the examinee to proceed from the last correctly connected letter. If the examinee completes the practice task with fewer than four errors, administer the Letter Sequencing condition. Say,

> **Good. Now let's try this one.**

Discontinue

Discontinue the scored Letter Sequencing condition after 150 seconds.

Note

If the examinee has difficulty holding the response booklet stationary, hold down the top or side edges of the booklet with your fingertips.

Condition 3: Letter Sequencing *(continued)*

Open the response booklet to the second and third pages. Place it flat on the table directly in front of the examinee, horizontally at the examinee's midline. Say,

> **Do this the same way by connecting *just the letters*. Begin at *A*** (point to the *A*) **and draw a line from *A* to *B*** (trace this connection with your finger), ***B* to *C*** (trace this connection with your finger), **and so on, in order, until you reach the end** (point to the *P*). **Draw the lines as quickly as you can without making mistakes. Ready? Begin.**

Start timing. If the examinee makes an incorrect connection, stop him or her immediately after he or she has completed the connection. Write an *X* over the incorrect connection, state that he or she has made an error, and, ***without explaining the error***, ask him or her to proceed from the last correctly connected letter. Keep the stopwatch running while pointing out errors.

When the examinee completes the last connection (reaches the letter *P*), stop timing. Record the total time in seconds on the record form.

If the examinee fails to finish the task by 150 seconds, say,

> **Stop. That's good.**

Allow the examinee to complete any connection in progress at the time limit before telling him or her to stop. This connection is scored as completed within the time limit.

Remove the Letter Sequencing Response Booklet.

Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Number–Letter Switching Response Booklet
- Pen (any color)
- Stopwatch

Discontinue

If the examinee cannot complete the practice task ***after four corrections*** by the examiner, discontinue the practice task and do not administer the scored Number–Letter Switching condition. Instead, proceed to Condition 5: Motor Speed.

Discontinue the scored Number–Letter Switching condition after 240 seconds.

Note

If the examinee has difficulty holding the response booklet stationary, hold down the top or side edges of the booklet with your fingertips.

Condition 4: Number–Letter Switching

Leave the stimulus booklet in its easel position. Place the Number–Letter Switching Response Booklet flat on the table and facing the examinee so that the examinee can easily write on the practice page. Point to the practice page and say,

> **This time, I want you to do something different. I want you to *switch* between connecting the numbers and letters. Begin at number one** (point to the 1) **and draw a line from one to *A*** (trace this connection with your finger), ***A* to two** (trace this connection with your finger), **two to *B*** (trace this connection with your finger), ***B* to three** (trace this connection with your finger), **and so on, in order, until you reach the end** (point to the *D*). **In other words, you will draw a line from a number to a letter, to a number, and so on, in order, until you reach the end. Do you have any questions? Draw the lines as quickly as you can without making mistakes. Go ahead.**

If the examinee makes an incorrect connection, stop him or her immediately after he or she has completed the connection. Write an *X* over the incorrect connection, explain the error, and point to the correct connection. Ask the examinee to proceed from the last correctly connected number or letter. If the examinee completes the practice task with fewer than four errors, administer the Number–Letter Switching condition. Say,

> **Good. Now let's try this one.**

Discontinue

Discontinue the scored Number–Letter Switching condition after 240 seconds.

Note

If the examinee has difficulty holding the response booklet stationary, hold down the top or side edges of the booklet with your fingertips.

Condition 4: Number–Letter Switching *(continued)*

Open the response booklet to the second and third pages. Place it flat on the table directly in front of the examinee, horizontally at the examinee's midline. Say,

> **On this page are more numbers and letters. Do this the same way by *switching* between numbers and letters. Begin at number one** (point to the 1) **and draw a line from one to *A*** (trace this connection with your finger), ***A* to two** (trace this connection with your finger), **two to *B*** (trace this connection with your finger), ***B* to three** (trace this connection with your finger), **and so on, in order, until you reach the end** (point to the *P*). **In other words, you will draw a line from a number to a letter, to a number, and so on, in order, until you reach the end. Draw the lines as quickly as you can without making mistakes. Ready? Begin.**

Start timing. If the examinee makes an incorrect connection, stop him or her immediately after he or she has completed the connection. Write an *X* over the incorrect connection, state that he or she has made an error, and, ***without explaining the error***, ask him or her to proceed from the last correctly connected number or letter. Keep the stopwatch running while pointing out errors.

When the examinee completes the last connection (reaches the letter *P*), stop timing. Record the total time in seconds on the record form. If the examinee fails to finish the task by 240 seconds, say,

> **Stop. That's good.**

Allow the examinee to complete any connection in progress at the time limit before telling him or her to stop. This connection is scored as completed within the time limit.

Remove the Number–Letter Switching Response Booklet.

Condition 5: Motor Speed

Place the Motor Speed Response Booklet flat on the table and facing the examinee so that the examinee can easily write on the practice page. Point to the practice page and say,

> **Here is a dotted line. I want you to start here** (point to "Start") **and draw a line over the dotted line as quickly as you can like this** (trace the first three connections with your finger). **Keep drawing over the dotted line until you reach the end** (point to "End"). **You do not have to draw your line neatly on the dotted line; just draw it as quickly as you can. Make sure your line touches every circle along the path. Do you have any questions? Go ahead.**

The following prompts may be given as often as necessary.

- If the examinee tries to draw over the dotted line as neatly as possible, thereby losing time, say, **Remember, it's more important to draw your line quickly than to make it neat.**
- If the examinee tries to take shortcuts and misses circles along the path, say, **Remember to make your line touch every circle along the path.**

If the examinee draws a line that departs from the dotted line and makes an incorrect connection or haphazard line, stop him or her immediately, explain the error, and redirect him or her to draw over the dotted line. If the examinee cannot complete the practice task after four corrections by the examiner, discontinue the practice task and do not administer the scored Motor Speed condition. If the examinee completes the practice task with fewer than four errors, administer the Motor Speed condition. Say,

> **Good. Now let's try this one.**

Open the response booklet to the second and third pages. Place it flat on the table directly in front of the examinee, horizontally at the examinee's midline. Say,

Test round - A3 sheet:

> **Again, I would like you to draw over the dotted line as quickly as you can. Start here** (point to "Start") **and draw a line like this** (trace over the first three connections with your finger) **until you reach the end** (point to "End"). **Remember, it's more important to draw the line quickly than to make it neat, but make sure your line touches every circle along the path. Ready? Begin.**

Start timing.

The following prompts may be given as often as necessary.

- If the examinee tries to draw over the dotted line as neatly as possible, thereby losing time, say, **Remember, it's more important to draw your line quickly than to make it neat.**
- If the examinee tries to take shortcuts and misses circles along the path, say, **Remember to make your line touch every circle along the path.**

If the examinee departs from the dotted line and makes an incorrect connection or haphazard line, stop him or her immediately and, ***without explaining the error***, redirect him or her to the dotted line. Keep the stopwatch running while pointing out errors.

When the examinee completes the path, stop timing. Record the total time in seconds on the record form. If the examinee fails to complete the path by 150 seconds, say,

> **Stop. That's good.**

Allow the examinee to complete any connection in progress at the time limit before telling him or her to stop. This connection is scored as completed within the time limit.

Remove the Motor Speed Response Booklet.

Appendix G

Delis-Kaplan Executive Function System (D-KEFS) Trail Making Test – Condition Four: Number-Letter Switching Scoring Procedure

Table G1

Scaled-Score Equivalents of Raw Scores by Each Age Group: Condition 4- Number-Letter Switching Completion Time

Table A.4. Scaled-Score Equivalents of Raw Scores by Each Age Group: Condition 4–Number–Letter Switching Completion Time

Scaled Score	Age Group 8	9	10	11	12	13	14	15	16–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	Scaled Score
1	—	—	>222	>207	>189	>161	>147	>143	>136	>148	>165	>175	>203	>237	—	—	1
2	—	240–238	222–210	207–196	189–178	161–152	147–139	143–135	136–129	148–140	165–156	175–165	203–191	237–224	240–238	—	2
3	240–238	237–232	209–196	195–183	177–166	151–142	138–130	134–126	128–120	139–130	155–145	164–153	190–178	223–209	237–223	240–238	3
4	237–233	231–218	195–182	182–170	165–155	141–132	129–121	125–117	119–111	129–121	144–134	152–142	177–165	208–194	222–208	237–233	4
5	232–218	217–204	181–169	169–158	154–142	131–122	120–112	116–108	110–103	120–112	133–124	141–131	164–152	193–180	207–193	232–218	5
6	217–203	203–189	168–155	157–145	141–130	121–112	111–103	107–99	102–94	111–102	123–113	130–119	151–139	179–165	192–178	217–203	6
7	202–198	188–175	154–141	144–132	129–119	111–102	102–94	98–90	93–85	101–93	112–102	118–108	138–126	164–150	177–163	202–198	7
8	197–173	174–161	140–128	131–120	118–107	101–92	93–85	89–81	84–77	92–84	101–92	107–97	125–113	149–136	162–148	197–173	8
9	172–158	160–146	127–114	119–107	106–95	91–82	84–76	80–72	76–68	83–74	91–81	96–85	112–100	135–121	147–133	172–158	9
10	157–143	145–132	113–100	106–94	94–84	81–72	75–67	71–63	67–59	73–65	80–70	84–74	99–87	120–106	132–118	157–143	10
11	142–128	131–118	99–87	93–82	83–72	71–62	66–58	62–54	58–51	64–56	69–60	73–63	86–74	105–92	117–103	142–128	11
12	127–113	117–103	86–73	81–69	71–60	61–52	57–49	53–45	50–42	55–45	59–49	62–51	73–61	91–77	102–88	127–113	12
13	112–98	102–89	72–59	68–56	59–49	51–42	48–40	44–36	41–33	44–37	48–39	50–39	60–48	76–62	87–73	112–98	13
14	97–83	88–75	58–46	55–44	48–37	41–32	39–31	35–27	32–25	36–28	38–29	38–29	47–35	61–48	72–58	97–83	14
15	82–68	74–60	45–32	43–31	36–25	31–22	30–21	26–18	24–16	27–18	28–18	28–18	34–22	47–33	57–43	82–68	15
16	67–53	59–46	31–18	30–18	24–14	21–12	20–12	17–9	15–7	17–7	17–7	17–9	21–9	32–18	42–28	67–53	16
17	52–38	45–32	17–6	17–6	13–3	11–3	11–3	<9	<7	<7	<7	<9	<9	17–4	27–13	52–38	17
18	37–23	31–17	<6	<6	<3	<3	<3	—	—	—	—	—	—	<4	<13	37–23	18
19	<23	<17	—	—	—	—	—	—	—	—	—	—	—	—	—	<23	19

Table G2

Scaled-Score Equivalents of Raw Scores by Each Age Group: Condition 4- Number-Letter Switching Total All Errors

Table A.19. Scaled-Score Equivalents of Raw Scores by Each Age Group: Condition 4–Number–Letter Switching Total All Errors

Scaled Score	Age Group 8	9	10	11	12	13	14	15	16–19	20–29	30–39	40–49	50–59	60–69	70–79	80–89	Scaled Score
1	>14	>10	>8	>8	>8	>6	>6	>6	>6	>6	>6	>6	>8	>10	>10	>14	1
2	14–13	10	—	8	8	—	—	—	—	—	—	—	8	10	10	14	2
3	12	9	8	—	7	6	6	6	6	6	6	6	7	9	9	13–12	3
4	11	8	7	7	6	5	5	5	5	5	5	5	6	8	8	11	4
5	10–9	7	6	6	—	—	—	—	—	—	—	—	—	7	7	10	5
6	8	6	5	5	5	4	4	4	4	4	4	4	5	6	6	9–8	6
7	7	5	4	4	4	3	3	3	3	3	3	3	4	5	5	7	7
8	6–5	4	—	3	3	—	—	—	—	—	—	—	3	4	4	6	8
9	4	3	3	—	2	2	2	2	2	2	2	2	2	3	3	5–4	9
10	3	2	2	2	1	1	1	1	1	1	1	1	1	2	2	3	10
11	2–1	1	1	1	—	—	—	—	—	—	—	—	—	1	1	2	11
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1–0	12
13	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	13
14	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	14
15	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	15
16	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	16
17	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	17
18	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18
19	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	19

Appendix H

Controlled Oral Word Association Test (COWAT)

VERBAL FLUENCY

Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Stopwatch

Discontinue

Do not discontinue. Administer all three trials.

For each trial, discontinue after 60 seconds.

Letter Fluency Prompts

- If the examinee fails to make a response after any 15-second interval, say, **Keep going.** Provide this prompt only once per trial.
- The first time an examinee generates three consecutive words that do not start with the designated letter, say, **The letter we are using now is _____.** Provide this prompt only once per trial.
- Keep the stopwatch running while providing prompts.

Condition 1: Letter Fluency

Place the stimulus booklet in its easel position. Say,

> **I'm going to say a letter of the alphabet. When I say begin, I want you to tell me as many words as you can that begin with that letter. You will have 60 seconds before I tell you to stop. None of the words can be names of people, or places, or numbers. For example, if I gave you the letter *T*, you could say *take, toy, tooth,* and so forth, but you should not say *Tom* because that is a person's name, you should not say *Texas* because that is the name of a place, and you should not say *twelve* because that is a number. Also, do not give me the same word with different endings. For example, if you say *take*, you should not also say *takes* or *taking*. Do you have any questions?**

Turn the page to display the summarized instructions to the examinee.

Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Stopwatch

Discontinue

Do not discontinue. Administer all three trials.

For each trial, discontinue after 60 seconds.

Letter Fluency Prompts

- If the examinee fails to make a response after any 15-second interval, say, **Keep going.** Provide this prompt only once per trial.
- The first time an examinee generates three consecutive words that do not start with the designated letter, say, **The letter we are using now is _____.** Provide this prompt only once per trial.
- Keep the stopwatch running while providing prompts.

Condition 1: Letter Fluency *(continued)*

Say,

> **Here is a page that will help you remember these rules.**

Review the instructions with the examinee, pointing to the four rules in turn. Leave the summarized instructions in the examinee's view throughout the administration of the Letter Fluency condition.

Note: If you are administering the Alternate Form of this test, substitute the letter that appears in brackets in the following instructions.

Trial 1

Say,

> **The first letter is *F* [B]. Ready? Begin.**

Start timing. On the record form, write the examinee's responses verbatim in the column labeled "F" ("B" if using the Alternate Form). Record responses that the examinee generates during the first 15 seconds in the first box (labeled "1–15 Seconds"), record responses given in the second 15 seconds in the second box (labeled "16–30 Seconds"), and so forth. After 60 seconds, say,

> **Stop.**

Trial 2

Introduce the letter by saying,

> **The next letter is *A* [H]. Ready? Begin.**

Start timing. Record the examinee's responses as described for Trial 1. After 60 seconds, say,

> **Stop.**

Trial 3

Introduce the letter by saying,

> **The next letter is *S* [R]. Ready? Begin.**

Start timing. Record the examinee's responses as described for Trial 1. After 60 seconds, say,

> **Stop.**

Condition 2: Categories

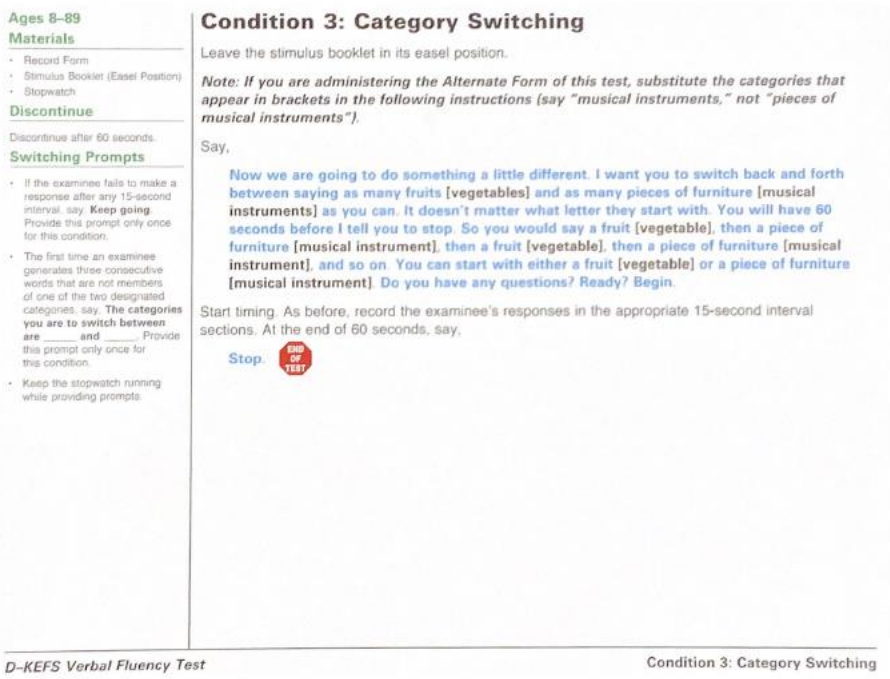
Animals:

Instructions:

Now I want you to do something similar. I want you to say as many different animals as you can. Name them as quickly as possible.

Allow ONE MINUTE for this test as well. If the patient discontinues before the end of the period encourage them to produce more names. Repeat the basic instructions and give the starting word 'dog' if there is a pause of 15 seconds or more. Start timing immediately after the instructions have been given, but allow extra time for the period when instructions are repeated. Write down the words in the order they were produced.

Scoring: Sum the number of admissible words for each letter and then total up the three amounts. Slang words and foreign words used as part of standard English are acceptable. Record the number of repetitions for each attempt. Also record made up words.
Scoring: Sum the number of admissible words. Names of extinct imaginary or magic animals are admissible but given names for animals (e.g. Fido) are not. Record the number of repetitions for each attempt. Also record made up words.



Ages 8–89

Materials

- Record Form
- Stimulus Booklet (Easel Position)
- Stopwatch

Discontinue

Discontinue after 60 seconds.

Switching Prompts

- If the examinee fails to make a response after any 15-second interval, say, **Keep going.** Provide this prompt only once for this condition.
- The first time an examinee generates three consecutive words that are not members of one of the two designated categories, say, **The categories you are to switch between are _____ and _____.** Provide this prompt only once for this condition.
- Keep the stopwatch running while providing prompts.

Condition 3: Category Switching

Leave the stimulus booklet in its easel position.

Note: If you are administering the Alternate Form of this test, substitute the categories that appear in brackets in the following instructions (say "musical instruments," not "pieces of musical instruments").

Say,

> Now we are going to do something a little different. I want you to switch back and forth between saying as many fruits **[vegetables]** and as many pieces of furniture **[musical instruments]** as you can. It doesn't matter what letter they start with. You will have 60 seconds before I tell you to stop. So you would say a fruit **[vegetable]**, then a piece of furniture **[musical instrument]**, then a fruit **[vegetable]**, then a piece of furniture **[musical instrument]**, and so on. You can start with either a fruit **[vegetable]** or a piece of furniture **[musical instrument]**. Do you have any questions? Ready? Begin.

Start timing. As before, record the examinee's responses in the appropriate 15-second interval sections. At the end of 60 seconds, say,

> Stop. END OF TEST

D-KEFS Verbal Fluency Test Condition 3: Category Switching

Appendix I

Beck Anxiety Inventory- Short Form (BAI-SF) & Highest Education Level

Please complete the following questionnaires which will ask you questions about your moods, and emotions, and whether or not you have noticed any problems with your memory.

Fill in your participant code here: _____

Please tick next to the box which best describes your highest level of education:

Primary school
High School
Matric
Undergraduate degree / diploma
Postgraduate degree

How did you sleep last night? Please circle:

Very badly Not so well Quite well Very well

Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today, by circling the number in the corresponding space in the column next to each symptom.

	Not at all	**Mildly, but it didn't bother me much**	**Moderately – it wasn't pleasant at times**	**Severely – it bothered me a lot**
Numbness or tingling	0	1	2	3
Feeling hot	0	1	2	3
Wobbliness in legs	0	1	2	3
Unable to relax	0	1	2	3
Fear of worst happening	0	1	2	3
Dizzy or lightheaded	0	1	2	3
Heart pounding / racing	0	1	2	3
Unsteady	0	1	2	3
Terrifed or afraid	0	1	2	3
Nervous	0	1	2	3
Feeling of choking	0	1	2	3

Hands trembling	0	1	2	3
Shaky / unsteady	0	1	2	3
Fear of losing control	0	1	2	3
Difficulty in breathing	0	1	2	3
Fear of dying	0	1	2	3
Scared	0	1	2	3
Indigestion	0	1	2	3
Faint / lightheaded	0	1	2	3
Face flushed	0	1	2	3
Hot / cold sweats	0	1	2	3

Appendix J

Beck Anxiety Inventory -Short Form (BAI-SF) Score Classifications

Table J

Beck Anxiety Inventory (BAI) Score Classifications

Severity of Anxiety	Total BAI Score
Minimal	0-7
Mild	8-15
Moderate	16-25
Severe	26-63

Note. Score Classifications as outlined by Beck & Steer (1993)

Appendix K

Beck Depression Inventory II (BDI-II)

This questionnaire consists of 21 groups of statements. Please read each group of statements carefully. And then pick out the one statement in each group that best describes the way you have been feeling during the past two weeks, including today.

Circle the number beside the statement you have picked.

1.	0	I do not feel sad
	1	I feel sad
	2	I am sad all the time and I can't snap out of it
	3	I am so sad and unhappy that I can't stand it
2.	0	I am not particularly discouraged about the future
	1	I feel discouraged about the future
	2	I feel I have nothing to look forward to
	3	I feel the future is hopeless and that things cannot improve
3.	0	I do not feel like a failure
	1	I feel I have failed more than the average person
	2	As I look back on my life, all I can see is a lot of failures
	3	I feel I am a complete failure as a person
4.	0	I get as much satisfaction out of things as I used to
	1	I don't enjoy things the way I used to
	2	I don't get real satisfaction out of anything anymore
	3	I am dissatisfied or bored with everything
5.	0	I don't feel particularly guilty
	1	I feel guilty a good part of the time
	2	I feel quite guilty most of the time
	3	I feel guilty all of the time
6.	0	I don't feel I am being punished
	1	I feel I may be punished
	2	I expect to be punished
	3	I feel I am being punished
7.	0	I don't feel disappointed in myself
	1	I am disappointed in myself
	2	I am disgusted with myself
	3	I hate myself
8.	0	I don't feel I am any worse than anybody else
	1	I am critical of myself for my weaknesses or mistakes
	2	I blame myself all the time for my faults

	3	I blame myself for everything bad that happens
9.	0	I don't have any thoughts of killing myself
	1	I have thoughts of killing myself, but I would not carry them out
	2	I would like to kill myself
	3	I would kill myself if I had the chance
10.	0	I don't cry any more than usual
	1	I cry more now than I used to
	2	I cry all the time now
	3	I used to be able to cry, but now I can't cry even though I want to
11.	0	I am no more irritated by things than I ever was
	1	I am slightly more irritated now than usual
	2	I am quite annoyed or irritated a good deal of the time
	3	I feel irritated all the time
12.	0	I have not lost interest in other people
	1	I am less interested in other people than I used to be
	2	I have lost most of my interest in other people
	3	I have lost all of my interest in other people
13.	0	I make decisions about as well as I ever could
	1	I put off making decisions more than I used to
	2	I have greater difficulty in making decisions more than I used to
	3	I can't make decisions at all anymore
14.	0	I don't feel that I look any worse than I used to
	1	I am worried that I am looking old or unattractive
	2	I feel there are permanent changes in my appearance that make me look unattractive
	3	I believe that I look ugly
15.	0	I can work about as well as before
	1	It takes an extra effort to get started at doing something
	2	I have to push myself very hard to do anything
	3	I can't do any work at all
16.	0	I can sleep as well as usual
	1	I don't sleep as well as I used to
	2	I wake up 1-2 hours earlier than usual and find it hard to get back to sleep
	3	I wake up several hours earlier than I used to and cannot get back to sleep.
17.	0	I don't get more tired than usual
	1	I get tired more easily than I used to
	2	I get tired from doing almost anything

	3	I am too tired to do anything
18.	0	My appetite is no worse than usual
	1	My appetite is not as good as it used to be
	2	My appetite is much worse now
	3	I have no appetite at all anymore
19.	0	I haven't lost much weight, if any, lately
	1	I have lost more than five pounds
	2	I have lost more than ten pounds
	3	I have lost more than fifteen pounds
20.	0	I am no more worried about my health than usual
	1	I am worried about physical problems like aches, pains, upset stomach, or constipation
	2	I am very worried about physical problems and it's hard to think of much else
	3	I am so worried about my physical problems that I cannot think of anything else
21.	0	I have not noticed any recent change in my interest in sex
	1	I am less interested in sex than I used to be
	2	I have almost no interest in sex
	3	I have lost interest in sex completely

Appendix L

Beck Depression Inventory II (BDI-II) Score Classifications

Table L

Beck Depression Inventory II (BDI-II) Score Classifications

Severity of Depression	Total BDI-II Score
Minimal	0-13
Mild	14-19
Moderate	10-28
Severe	29-63

Note. Score Classifications as outlined by Beck et al. 1996

Appendix M

Information Sheet & Informed Consent Form

APPLIED MICROBIAL AND HEALTH BIOTECHNOLOGY INSTITUTE, CAPE PENINSULA UNIVERSITY OF TECHNOLOGY, BELLVILLE CAMPUS, BELLVILLE SOUTH AFRICA

Participant Information Sheet and Informed Consent Form

This Informed Consent Form is for men and women who we are invited to participate in research on Rooibos and its potential heart health benefits. The title of our research project is "Rooibos, Heart and Cognitive Health"

Name of Study leader: Prof JL Marnewick
Name of Organization: Cape Peninsula University of Technology (CPUT)
Name of Sponsor: Cape Peninsula University of Technology - Prestigious Project Funding & South African Rooibos Council
Name of Project proposal: Oxidative Stress in Health and Disease: a Rooibos perspective

This Informed Consent Form has two parts:

- **Information Sheet (to share information about the research with you)**
- **Certificate of Consent (for signatures if you agree to take part)**

You will be given a copy of the full Informed Consent Form

PART I: Information Sheet

Introduction
I am Prof JL Marnewick, Director of the Applied Microbial and Health Biotechnology Institute of CPUT. We are doing research to establish the health promoting properties of Rooibos, which is an indigenous herbal tea in South Africa. I am going to give you information and invite you to be part of this research. There may be some words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain it. If you have questions later, you can ask them of me, or any member of the study team present here today. You do not have to decide today whether or not you will participate in the research study.

Purpose of the research
Oxidative stress is involved in the development of various chronic diseases such as heart disease, cancer and diabetes and in cognitive/mental health, all important and relevant in the South African context. Exhausted antioxidant defenses in the human body together with an inadequate intake of antioxidant-rich foods & beverages, contribute to oxidative stress and inflammation. When accumulated (as with ageing), your heart health and cognitive health may be affected. The current research is proposed to address this issue by focusing on the indigenous

herbal tea, rooibos, as an innovative measure to reduce the oxidative stress-induced damage in your body.

"A good case can be made for the notion that health depends on a balance between oxidative stress and antioxidant defenses." – quote by Dr Andrew Weill, M.D.

Type of Research Intervention
This research will involve you to drink 3 capsules containing either a freeze-dried water extract of traditional Rooibos tea (red) or of Green Rooibos tea or a placebo every day with your food for a period of twelve (12) weeks. The content of one capsule is equivalent to 2 cups of rooibos tea. Blood samples will be taken on two (2) occasions from your forearm during this study period. You will also be required to provide two (2) 24-hr urine samples during this study period.

Participant selection
We are inviting all adults within the age range of 30 to 80 years old who meet certain criteria to take part in the research study. The inclusion criteria for participants will include a stable body weight 6 months before the study starts, female participant not pregnant or lactating, consumption of less than 2 alcoholic beverages/day, following a conventional diet and not a special diet (i.e. vegetarian), absence of cardiovascular disease, diabetes, renal, hepatic and endocrine disorders and not taking any medication, vitamins or dietary supplements with established antioxidant properties. In addition, participants should present with any two or more of the following risk factors: hyperlipidemia (raised cholesterol - >5.5-7.7 mmol/L), pre-hypertension (120-139/80-90 mm Hg), smoking, increased BMI (>25 to 38 kg/m^2), but not requiring any medication for these medical conditions and family history of cardiovascular disease and/or Mental health/Alzheimer's disease.

Voluntary Participation
Your participation in this research study is entirely voluntary. It is your choice whether to participate or not. Even if you have agreed to take part in the study, you may still stop participating at any time you want without any consequences to you.

Procedures and Protocol
This project forms part of a human intervention study to determine the effect of rooibos consumption on antioxidant/oxidative stress, inflammation and certain genetic measures in the blood of volunteers. There are no known risks to participate in this study. If you volunteer to take part in this study, you will be asked to do the following:

1) Answer questions and complete questionnaires about my demography, health, diet/food intake, dietary supplements, physical activity and mental health. The study nurse will also take my body measurements, (such as height, weight, and waist circumference) at 2 (two) occasions during the study.
2) Take part in a study over a 14 (fourteen) week period which includes the completion of a self-administered dietary record, based on my habitual dietary intake for the first 14 days. During this 2-week period (known as the *wash-out period*) you will be requested to follow a flavonoid-restricted, omit/restrict certain antioxidant-containing food and beverages from my diet and continue to complete the dietary records. The following 12 (twelve) weeks is the *intervention period* in which you will consume 3 capsules daily with meals (one capsule with every meal), follow the same flavonoid-restricted diet and once again complete the dietary food records. You will receive training at the beginning

of the study on how to complete the dietary records, flavonoid frequency questionnaire and the health questionnaire.

3) If you are willing, a qualified phlebotomist or nursing sister will take samples of your blood from your forearm (6 blood tubes), take your blood pressure measurements, your body measurements, while trained professionals will examine & evaluate the structures of your eyes, as well as do an ultrasound scan of your neck and chest area and ask you certain questions while measuring the response in your eyes. These procedures will be done on 2 (two) occasions. The first occasion (1st visit) will be after you have followed the 14-day wash-out period and will serve as your baseline. The second occasion (2nd visit) will be after you have completed 12 weeks of taking 3 capsules daily. Blood and urine samples will be analysed to determine your general health, blood lipids, antioxidant/oxidative stress status, inflammatory& stress status, and related biochemistry status as well as include your metabolomic & genetic profiling with respect to coronary heart disease, the metabolic syndrome and Cognitive health. Individuals with different genetic profiles react differently to dietary and lifestyle factors and genetic testing may explain why only a subset of the study participants may benefit from the rooibos intervention.
4) The questions and blood tests are not for diagnostic purposes, your blood will not be tested for HIV-AIDS. Should the study doctor deem it necessary after your blood test results are known, he will refer you to your personal physician or local clinic doctor.
5) To also supply 2 (two) 24 hour-urine samples on the same time points as the blood samples for antioxidant content, oxidative stress and related biochemistry analyses.
6) Someone from the study may call you to clarify your information.

All these above procedures will probably be familiar to you as most of them occur when you visit your general practitioner, radiographer and optometrist.

Randomization

Because we do not know if the rooibos capsules will be better, we need to compare it to a placebo capsule (containing no antioxidants). To do this, we will place people taking part in this research into three groups. The groups are selected by chance, as if tossing a coin, also called *randomization.* Participants in one group will be given the green rooibos capsule while participants in the other two groups will be given the fermented rooibos capsule or the placebo capsule. The placebo or inactive capsule will look like the rooibos capsule, but does not contain the active substances. It is important that neither you nor we know which of the three capsules you are given. This information will be in our files, but we will not look at these files until after the research is finished or unless deemed necessary by the study doctor. This is the best way we have for testing without being influenced by what we think or hope might happen. We will then compare which of the three has the best results.

Side Effects

We do not anticipate any side affects you may experience, but should you experience any unease or feel unsure during the study please contact the study doctor, Dr Kobus Pretorius (cell number/WhatsApp 084 9717 637) or study coordinator Sr Nola Baartman (cell number/ WhatsApp 082 876 1064) immediately and they will advise and guide you in this regard.

Risks

No risk is expected but you may experience some discomfort when your blood is drawn or when the researchers ask you questions about your general and mental health, nutrition, physical activity and smoking habits. The risks of drawing blood from your arm include the

unlikely possibilities of a small bruise or localized infection and bleeding. These risks will be reduced by using a qualified phlebotomist or nursing sister to draw the blood.

Benefits

Apart from getting to know certain of your health indicators such as blood pressure, cholesterol and blood glucose levels, there are not any direct benefit for you but your participation is likely to help us find the answer to the research questions.

Costs

You will not be given any money or gifts to take part in this research study nor will you be charged any costs for the results of your blood cholesterol and glucose and blood pressure.

Confidentiality

Your personal information we collect during this research study will be kept confidential, it will be stored in a locked filing cabinet and on a password protected computer to which only the study leader, Prof JL Marnewick and study coordinator, Sr Nola Baartman will have access. Any information about you will have a number on it instead of your name. Only the study leader will know what your number is, it will not be shared with or given to anyone except Dr Pretorius, the study doctor, if and when deemed necessary.

Sharing the Results

The knowledge that we get from doing this research will be shared at public meetings such as conferences and we will also publish the results in order that other interested people may learn from our research. Confidential information will not be shared. You should note that this is a long process and may take up to 4 years after the study has been completed.

Right to Refuse or Withdraw

You do not have to take part in this research if you do not wish to do so. You may also stop participating in the research at any time you choose. It is your choice and all of your rights will still be respected.

Who to Contact?

If you have any questions, you may ask them now or later, even after the study has started. If you wish to ask questions later, you may contact the study coordinator, Sr Nola Baartman at cell/WhatsApp 082 876 1064 or email: Rooibostrial@gmail.com or the study leader, Prof JL Marnewick at cell/WhatsApp 082 897 9352 or email marnewickj@cput.ac.za at the Applied Microbial and Health Biotechnology Institute of the Cape Peninsula University of Technology, Bellville Campus, Corner of Symphony way and Robert Sobukwe Drive, Bellville East, Bellville).

How to take your Study capsules?

- Take 1 capsule after every meal per day (one capsule after breakfast, one capsule after lunch and one capsule after dinner).
- Total of 3 capsules taken with food throughout the day.
- Take for 12 weeks continuously without skipping any one of the three occasions daily.
- Please return all capsule holders at the end of the 12 weeks for record purposes

This research study has been approved by the Faculty of Health and Wellness Research Ethics Committee (H&W REC); a committee tasked to make sure that research participants are protected from harm. If you wish to find more about the H&W REC, contact The Chairperson, H&W REC, Tel: +27 21 959 6917; email: sethn@cput.ac.za.

PART II: Certificate of Consent

Declaration by study participant:

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this research. I may choose to leave this study at any time and will not be penalised or prejudiced in any way. I may be asked to leave the study before it has finished if the study doctor or study leader feels it is in my best interest or if I do not follow the study plan as agreed to.

Place_____

Print Name of Participant_____

Signature of Participant _____

Date _____
Day/month/year

Signature of witness_____

Declaration by investigator:

I declare that I have explained the information in this document to the study participants and encourage them to ask questions and took adequate time to answer them. I am satisfied that he/she adequately understands all aspects of the research study as discussed above. I did/did not use an interpreter. (If an interpreter is used the interpreter must sign the declaration below).

Place_____

Print Name of Investigator_____

Signature of Investigator _____

Date _____
Day/month/year

Signature of witness_____

Declaration by interpreter:

I declare that I assisted the investigator to explain the information in this document to the participant using the language medium of Afrikaans/Xhosa. We encouraged him/her to ask questions and took adequate time to answer them. I conveyed a factual correct version of what was related to me. I am satisfied that the participant fully understands the content of this

information and informed consent document and has had all his/her questions satisfactorily answered.

Place_____

Print Name of Interpreter_____

Signature of Interpreter _____

Date _____
Day/month/year

Signature of witness_____

 **Thumb print of participant**

_____

In line with the Protection of Personal Information Act (POPIA), I understand the purpose for which my personal information will be collected and hereby give written consent for my information to be collected and stored in terms of this purpose".

Signed at:

Signature of participant:

Date:

_____

INFORMATION ON HOW TO COLLECT YOUR 24-hour URINE SAMPLE

IMPORTANT: This sample you will need to collect the day BEFORE you have your scheduled laboratory visit

1. **On the day before your laboratory visit, urinate into the toilet when you get up in the morning.**

2. **Then collect all your urine for that day in the special Big container supplied to you for the next 24 hours.**

3. **On the morning of your laboratory visit, urinate into the Big container when you get up in the morning.**

4. **Cap the Big container, invert the closed container and then pour a sample of the mixed 24-hour urine you have collected into the Smaller container you were supplied with.**

5. **Please only bring the Smaller container with you that day when you come to the laboratory on campus.**

Smaller urine container is filled from the bigger urine container**.**

INFORMATION FOR PARTICIPANTS HAVING A CAROTID ARTERY ULTRASOUND

This leaflet aims to answer your questions about having a Carotid Artery Ultrasound scan. It explains the benefits, risks as well as what you can expect. If you have any further questions, please ask the nursing sister available.

What is an ultrasound scan?
An ultrasound machine uses high frequency sound waves to image parts of the human body including blood vessels. This study will use such a machine to look at the arteries in your neck for wall thickening as well as plaque formation.

What happens during an ultrasound scan?
This ultrasound examination takes about 30-40 minutes. It is a painless examination and requires you to lie on your back while a small instrument called a probe is moved across the skin. A water-based gel is first applied to the skin so the probe has good contact and can move smoothly over the skin. Gel which might get onto your clothes will wash out easily. Some patients find this test relaxing. Images of the neck arteries are captured onto the Ultrasound system and reviewed by the sonographer.

What are the risks?
There are no known risks to ultrasound imaging. Please advise the staff if you may be allergic to the silicone gel.

How can I prepare for my ultrasound scan?
Ensure that you wear loose fitting clothes around your neck area to allow adequate access for the scan, for example avoid polo neck jerseys or neck ties. Please remove all jewellery such as earrings, studs or necklaces before the scan. You will be provided with a disposable gown.

Will I feel pain?
This procedure is not painful, but you will need to remain still for the duration of the scan. The gel may feel a bit cold and wet.

What happens after I have had the ultrasound scan?

- After the examination you will be able to return to work or home
- You should have no after-effects
- You can eat and drink and carry on with all your normal activities

If you have any questions, please speak to our nursing sister or study staff.

INFORMATION FOR PARTICIPANTS HAVING RETINAL IMAGING PROCEDURES

This leaflet aims to answer your questions about having retinal imaging procedures. It explains the benefits, risks as well as what you can expect. If you have any further questions, please ask the consultant available.

What is retinal imaging?
Images of the back of your eye may be recorded by using fundus photography or Optical Coherence Tomography (OCT). Fundus photography uses a conventional digital camera attached to a focusing system that allows photographs to be taken through your pupils. Optical coherence tomography provides three dimensional or cross-sectional structural images of the eye and retina based on back-reflected light. OCT angiography is a further process that uses software in the computer to analyse the blood vessels and to detect changes or abnormalities in their structure and the flow of blood in the back of the eye.

What happens during retinal imaging procedures?
Fundus photography can be performed in less than five minutes and the OCT scans can be completed in about ten minutes. Both procedures are non-invasive and painless. You will be seated comfortably at either instrument and will be asked to place your head in a chin rest against a forehead rest. During fundus photography you will experience a bright flash of light just like you would when a camera flash goes off. For OCT you will be asked to focus on a light and will not experience any discomfort. In both procedures the equipment will scan your eye without touching it.

What are the risks?
There are no known risks to fundus photography or optical coherence tomography.

How can I prepare for these imaging procedures?
Ensure that you wear loose fitting clothes around your head and neck area to allow easy access to the imaging instruments. No additional preparations should be necessary.

Will I feel pain?
You will not feel any pain or discomfort although the bright flash of light may dazzle your eyes for a few moments.

What happens after I have had the retinal scans?

- After the examination you will be able to return to work or home
- You should have no after-effects
- You can eat and drink and carry on with all your normal activities

If you have any questions, please speak to our study staff.

INFORMATION FOR PARTICIPANTS HAVING AUTOREFRACTION AND NON-CONTACT TONOMETRY

What is autorefraction?
The prescription (that would usually be what is used to make your spectacles) of your eyes may be measured using a machine called the Autorefractor. In addition, this instrument can also record the curvature of the eye as well as the thickness of the cornea of the eye. The cornea is the clear front part of the eye in front of the pupil and coloured iris.

What is non-contact tonometry?
The same instrument used to measure your prescription and corneal curvature and thickness can be used to measure the pressure inside the eye. The measurement of the pressure inside the eye can be taken right after the instrument records your prescription. The purpose of measuring the pressure inside the eyes is to check whether the eye is at risk of developing glaucoma. Although glaucoma can occur in eyes with normal pressure, high pressure inside the eyes is a known risk factor for glaucoma.

What happens during autorefraction and non-contact tonometry?
Autorefraction and non-contact tonometry can be measured all at once in less than five minutes. Both procedures are non-invasive and painless, although the puff of air that occurs during non-contact tonometry can feel a little unusual and uncomfortable for a few seconds. For both procedures you will be seated comfortably at the instrument and will be asked to place your head in a chin rest against a forehead rest. During autorefraction you will be asked to look at a picture in the machine and then the instrument will automatically measure your prescription. The picture may go blurred and then come back into focus. After this the machine will blow a short puff of air onto your eye. In both procedures the equipment will scan your eye and perform its operation without touching the eye at all.

What are the risks?
There are no known risks for autorefraction or non-contact tonometry.

How can I prepare for these imaging procedures?
Ensure that you wear loose fitting clothes around your head and neck area to allow easy access for the instrument. No additional preparations should be necessary.

Will I feel pain?
You will not feel any pain although you may feel a little discomfort for a few seconds when the puff of air blows onto your eye. The operator will demonstrate this to you.

What happens after I have had autorefraction and non-contact tonometry?
- After the examination you will be able to return to work or home
- You should have no after-effects
- You can eat and drink and carry on with all your normal activities
- If the operator finds anything unusual with the results of your eye, you will be referred for further examination by the study doctor.

If you have any questions, please speak to our study staff.

INFORMATION FOR PARTICIPANTS UNDERGOING PUPIL DIAMETER, DILATION AND SPONTANEOUS EYE-BLINK MEASURES AND HEART RATE VARIABILITY (HRV) ASSESSMENT

What is pupil diameter, dilation and spontaneous eye-blink?

In order to facilitate accurate vision, the pupils in our eyes change size in response to light and moving objects, but they can also change when we concentrate. The diameter is the size of the pupil at any given time. Dilation refers to the widening of the pupil. When our eyes automatically shut closed in a reflex reaction, this is referred to as 'spontaneous eye-blink'. The term 'pupillometry' is used to refer to the measurement of these actions of the pupil.

What happens during pupil diameter, dilation and spontaneous eye-blink measurement?
You will be fitted with a pair of glasses that has a small camera attached to it, which will record changes in the size of your pupil. During this measurement, the camera will record the response of your pupil in response to 1) a flash of light, and 2) a cognitive task in which we ask you to repeat lists of numbers.

What is heart rate variability?
Heart rate changes over time in response to physical activity and emotions. Heart rate variability refers to the different lengths of intervals between each heartbeat when it heart rate is measured over a period of time. Heart rate variability is thought to reflect, in general, the ability of the heart to respond to a variety of challenges that you might face in any given day and many researchers use it to measure stress levels.

What happens during heart rate variability measurement?
To measure your heart rate variability, you will be fitted with a soft textile strap around your upper arm. Sensors on the strap will detect your heart beat and record this data. You will be fitted with the heart rate strap prior to the pupil response measure and it will record your heart rate for the duration of that task.

What are the risks?
There are no risks to undergoing these procedures.

How can I prepare for these procedures?
Ensure that you wear a loose-fitting top so that your sleeve can be pushed back to fit the heart rate strap around your upper arm.

Will I feel pain?
You will not feel pain during these procedures.

What happens after I have had the pupil dilation and heart rate variability tests?

- After the examination you will be able to return to work or home
- You should have no after-effects
- You can eat and drink and carry on with all your normal activities

If you have any questions, please speak to our study staff.

Appendix N

Dietary Restrictions List

Cape Peninsula University of Technology

Dietary restrictions for the Rooibos Intervention study 2021

Study participants are kindly asked to **strictly adhere to the following beverage and food intake restrictions** during the study, starting with the 2-week wash-out period.

1. Beverages

Restrict the intake of the following flavonoid-containing beverages to one cup/glass per day while participating in this study, as it may influence the outcome of the study.

- ***Coffee*** (all brands: Pure and instant; Caffeinated and decaffeinated; Filter, percolated or plunger)
- ***Cocoa drinks*** (all brands e.g. Hot chocolate, Milo, Ovaltine)
- Red wine; Rosé (all labels)
- **Fruit juices** – 100% pure juices and blends (all brands of red grape, orange, apple and berry juices along with red Grapetizer and Appletizer) = ½ **cup or 125 mL**
- **Fruit juices** (specific flavors: Litchi, White grape, Hanepoot), fruit nectars (≤ 50% fruit juice), dairy fruit blends and fruit juice and yoghurt blends = ½ **cup or 125 mL**

The following beverages may be taken in **restricted quantities per day**:

- ***Soda drinks***: Coca cola, Coke Light, Tab, Fanta, Cream Soda, Sprite, Lemon Twist (one 340 mL can per day)
- ***Two glasses of white wine*** (120 mL per glass) or ***one beer*** (340 mL) or ***one spirit*** drink (1 tot or 25 mL) per day

The following beverages may be taken as **usual/freely per day**:

- Diluted base drinks (specific brands: OROS, Jive, Tang, Drink-O-Pop, Sweet-O)
- ***Water*** / Bottled / Mineral (all brands: Still and sparkling; Flavored and unflavored)

2. Fruits

Restrict the number of portions consumed **per day** of the following fruits:

- ***Apples or Pears*** (one apple or one pear per day)
- ***Oranges or naartjies*** (one orange or one naartjie per day)
- ***Black/Red grapes or berries*** (including black berries, blue berries and strawberries (one cup per week)

All other fruits (e.g. banana, peach) may be consumed **as usual/freely**.

3. Dark Chocolate

Restrict the intake of ***dark chocolate*** to a maximum of three 40g (about 6 blocks) portions **per week**. All other chocolate (milk) may be consumed **as usual/freely.**

4. Sorghum – **Avoid** cereal & cereal products containing **sorghum** (e.g. maltabella porridge)

5. Dietary supplements

No vitamin C supplementation or any other antioxidant supplement (capsules or powder) should be taken during the study period as it may influence the outcome of the study.

NOTE: ***Study participants are also kindly asked not to change any other aspect regarding their food and beverage intakes during the study period compared to before the study***.

Appendix O

Health Measurements and Procedure (used in CPUT study)

1. Fasting (10–12 h) peripheral venous blood samples (volume of ~ 32-40 mL) were collected into two SST tubes and two EDTA blood tubes (BD vacutainers, Plymouth, UK) for serum and plasma preparation. The samples were protected from light and kept/ transported on ice to the laboratory for processing the same day. Blood samples were centrifuged (1000×g, 10 min, 4 0C) to obtain plasma and serum and stored at −40 0C until analyzed. Whole blood samples for oxidized glutathione (GSSG) analysis were treated with 30mM of 1-methyl-2-vinylpyridinium trifluoromethanesulphonate, M2VP, purchased from Merck, SA, before stored at −80 0C.
2. Anthropometric measurements (height, weight and waist circumference)
3. Blood pressure readings were taken using an automatic blood pressure instrument (Rossmax MV701i, Rossmax International, Ltd., Taiwan). Participants were required to relax for 5min before three blood pressure readings,(1 min apart) were taken on the same mornings as the fasting blood samples were collected.
4. Sonar of neck arteries (using an ultrasound probe)
5. Sonar of heart (using a transducer)
6. Eye measurements (taken via photographs of the eye blood vessels)
7. Changes in pupil size (recorded via a pupilometer)

All physical and health measurements, tests, and procedures were done by qualified phlebotomists and/or nursing sisters.

Appendix P

Abbreviated Food Frequency Questionnaire

Respondent number

SCREENING QUESTIONNAIRE – CONSUMPTION OF FOODS RICH IN FAT

South African Medical Association Dyslipidaemia Nutrition Working Group (2000:185)

Instructions for completion:
Think about your eating habits over the past year or so. Approximately how often do you eat each of the following foods? Mark an "**x**" in one box for each food.

Meat and snack intake		0 **Never or once or less than once per month**	1 **2 – 3 times per month**	2 **1 – 2 times per week**	3 **3 – 4 times per week**	4 **5 + times per week**	For office use only
1	Hamburgers or cheeseburgers						
2	Red meat, e.g. beef and mutton						
3	Fried chicken (with skin)						
4	Hot dogs, frankfurters, salami, Russians, sausages						
5	Cold cuts, lunch meats, ham (with fat), etc.						
6	Salad dressings, mayonnaise, etc.						
7	Margarine or butter						
8	Eggs						
9	Bacon or pork sausage						
10	Cheese or cheese spread						
11	Full-cream milk						
12	Potato chips ('slap chips')						
13	Potato crisps, corn chips, popcorn, etc.						
14	Ice-cream						
15	Doughnuts, cakes, cookies, puddings, etc.						
						For office use only	

Appendix Q

Ethics Certificate

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

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REC Approval Reference No:
CPUT/HW-REC 2017/H9- extension

Faculty of Health and Wellness Sciences

Dear Professor Marnewick

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC on 30 March 2017 to Professor Jeanine Marnewick for ethical clearance. This approval is for research activities related to research for Professor Marnewick at Cape Peninsula University of Technology – Institute of Biomedical and Microbial Biotechnology. An amendment was considered and approved to a new COVID-19 assay to the project, in May 2023.

TITLE: Rooibos and Heart Health, within the proposal titles "Oxidative stress in health and disease: a rooibos perspective"

Comment: The addendum request to add a voluntary COVID-19 test for study participants, using saliva samples has been considered and approved.

Approval will not extend beyond 11 May 2023. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Ms Carolynn Lackay
Chairperson - Research Ethics Committee
Faculty of Health and Wellness Sciences

Appendix R

Debriefing

Debriefing was sent in the form of an SMS to all participants. This SMS thanked participants for their time and contribution to the study. It let participants know that when the study results are published a jargon free summary would be sent to them. Participants were informed that their results were anonymous and confidential and that no-one would be able to identify them from their collected data. The SMS re-emphasized the study aims, procedures, incentives, risks, and gain explained why double-blinding and randomization were used, as outlined in the information, and informed consent form (see Appendix M). They were again provided with the contact details of the study leader/coordinator if they needed to contact them with questions relating the study/ their participation.

If this sub-study were to send a debriefing email it would read as follows:

A study investigating the effects of Fermented and Green Rooibos on cognition and mood in South African adults at risk for cardiovascular disease

This study was designed to examine the effects of Green and/or Fermented Rooibos on mood and cognition. The study was run over 12 weeks, and during this time participants consumed 3 capsules of either Green Rooibos, Fermented Rooibos, or a placebo per day. Previous studies have suggested that the rooibos may have health promoting properties and could help improve cognition and mood.

Both participants and the researchers did not know which intervention each participant received to accurately assess the effects of rooibos on mood and cognition.

Thank you for your time, participation and contribution to science

Appendix S

3 x 2 Factorial ANOVA Outputs for Cognitive Measures

Table S

Type III ANOVA results using cognitive measures as criterion:

Criterion	Predictor	Sum of Squares	*df*	*Mean Square*	*F*	*p*	η^2	$_{partial}\eta^2$
D-KEFS Trail								
Switch time	Group	10.98	2	5.49	1.18	0.311	0.010	0.012
	Session	**37.36**	**1**	**37.36**	**8**	**0.005****	**0.034**	**0.04**
	HLE	**132.85**	**3**	**44.28**	**9.49**	**0.000*****	**0.120**	**0.132**
	Sex	**36.04**	**1**	**36.04**	**7.72**	**0.006****	**0.033**	**0.040**
	BMI	3.65	1	3.65	0.78	0.378	0.003	0.004
	Chol	0.19	1	0.19	0.04	0.842	.000	.000
	Age	6.94	1	6.94	1.49	0.224	0.006	0.008
	Group: Session	2.63	2	1.31	0.28	0.755	0.002	0.003
Switch Errors	Group	8.77	2	4.39	0.93	0.396	0.007	0.008
	Session	8.37	1	8.37	0.78	0.184	0.007	0.008
	HLE	**50.98**	**3**	**16.99**	**3.61**	**0.014***	**0.043**	**0.046**
	Sex	9.34	1	9.34	1.85	0.160	0.008	0.009
	BMI	3.94	1	3.94	0.84	0.361	0.003	0.004
	Chol	3.98	1	3.98	0.83	0.364	0.003	0.004
	Age	11.15	1	11.15	2.37	0.125	0.009	0.010
	Group: Session	**29.48**	**2**	**14.74**	**3.13**	**0.046***	**0.003**	**0.027**
RAVLT								
Recognition	Group	2.56	2	1.28	0.27	0.765	0.002	0.002
	Session	4.75	1	4.75	0.1	0.319	0.004	0.005
	HLE	**50.97**	**3**	**16.99**	**3.57**	**0.016***	**0.044**	**0.047**

Criterion	Predictor	Sum of Squares	*df*	*Mean Square*	*F*	*p*	η^2	$_{partial}\eta^2$
	Sex	**20.1**	**1**	**20.1**	**4.24**	**0.04***	**0.018**	**0.019**
	BMI	0.06	1	0.06	0.01	0.909	.000	.000
	Chol	0.07	1	0.07	0.02	0.901	.000	.000
	Age	0.1	1	0.1	0.02	0.883	.000	.000
	Group: Session	**32.68**	**2**	**16.34**	**3.43**	**0.035***	**0.028**	**0.031**
Delayed Recall	Group	5.47	2	2.73	0.98	0.378	0.008	0.011
	Session	**102.67**	**1**	**102.67**	**36.74**	**0.000*****	**0.145**	**0.171**
	HLE	**66.35**	**3**	**22.12**	**7.91**	**0.000*****	**0.093**	**0.118**
	Sex	**34.98**	**1**	**34.98**	**12.52**	**0.001*****	**0.049**	**0.066**
	BMI	0.02	1	0.02	0.01	0.933	.000	.000
	Chol	0.78	1	0.78	0.28	0.598	0.001	0.002
	Age	2.42	1	2.42	0.87	0.354	0.003	0.005
	Group: Session	1.06	2	0.53	0.19	0.827	0.001	0.002
COWAT	Group	3.06	2	1.53	0.03	0.97	.000	.000
	Session	**825.12**	**1**	**825.12**	**16.19**	**.000*****	**0.065**	**0.084**
	HLE	**2491.5**	**3**	**830.5**	**16.29**	**.000*****	**0.195**	**0.217**
	Sex	163.07	1	163.07	3.2	0.075	0.013	0.018
	BMI	143.53	1	143.53	2.82	0.095	0.011	0.016
	Chol	32.11	1	32.11	0.63	0.428	0.003	0.004
	Age	6.74	1	6.74	0.13	0.716	0.001	0.001
	Group: Session	91.95	2	45.98	0.9	0.478	0.007	0.010
DSF	Group	3.186	2	1.54	1.02	0.362	0.008	0.01
	Session	**13.43**	**1**	**13.43**	**8.62**	**0.004****	**0.025**	**0.043**
	HLE	**65.25**	**3**	**21.75**	**13.96**	**.000*****	**0.169**	**0.178**
	Sex	0.00	1	0.00	0.003	0.959	.000	.000

Criterion	Predictor	Sum of Squares	*df*	*Mean Square*	*F*	*p*	η^2	$_{partial}\eta^2$
	BMI	0.62	1	0.62	0.339	0.528	0.002	0.002
	Chol	0.97	1	0.97	0.623	0.431	0.003	0.003
	Age	1.46	1	1.46	0.935	0.335	0.004	0.005
	Group: Session	1.22	2	0.112	0.072	0.931	.0001	.001
DSB	Group	0.8	2	0.4	0.35	0.704	0.003	0.004
	Session	0.7	1	0.7	0.62	0.432	0.003	0.003
	HLE	**25.12**	**3**	**8.37**	**7.38**	**.000*** **	**0.1**	**0.106**
	Sex	**3.44**	**1**	**3.44**	**3.03**	**0.083 .**	**0.014**	**0.016**
	BMI	1.5	1	1.5	1.32	0.252	0.006	0.007
	Chol	0.98	1	0.98	0.87	0.353	0.004	0.005
	Age	1.46	1	1.46	1.29	0.258	0.006	0.007
	Group: Session	5.65	2	2.82	2.49	0.086	0.022	0.026

Note. D-KEFS Trail = Delis Kaplan Executive Function System Trail Making Test: Switch Time = Switch Time= Number-Letter Switching completion time; Switch Errors = Number Letter Switching errors; RAVLT= Ray Auditory Verbal Learning Test; COWAT= Controlled Oral Word Association; DSF= Digit Span Forwards; DSB= Digit Span Backwards.

Group = Intervention group: Fermented Rooibos, Green Rooibos, and Placebo

Session = Time of measurement: Baseline and Follow-up

HLE = Highest Education level; BMI= Body Max Index.

Cholesterol and BMI were used as controls for cardiovascular disease risk in the analysis.

η^2 = Eta Squared; $_{partial}\eta^2$ =Partial Eta Squared

$\eta^2$and $_{partial}\eta^2$ were used as measure of effect sizes

*p<0.01. ** p<0.001. *** p<0.000. '.' $p<0.05$

Appendix T

Significant ANOVA findings for cognition: descriptive statistics

Table T1

Descriptive Statistics for Significant Pre/Post Intervention Changes in Cognitive Scores

Measure	Baseline	Follow-up
	Mean±SD	Mean±SD
D-KEFS TST	7.21 ±4.13	7.89 ±3.96
COWAT	60.37 ±16.72	57.17± 17.16
RAVLT: Delayed Recall	8.79 ±3.45	7.65 ±3.48
DSF	8.6 ±2.07	9.01 ± 2.23

Note. D-KEFS TST = Delis Kaplan Executive Function System Trail Making Test: Number-Letter Switching completion time; COWAT= Controlled Oral Word Association; RAVLT= Ray Auditory Verbal Learning Test; DSF= Digit Span Forwards

Table T2

Descriptive Statistics of Cognitive Scores per Highest Education Level

Measure	Primary School	High-School	Undergraduate	Post-graduate
	Mean±SD	Mean±SD	Mean±SD	Mean±SD
D-KEFS Trail				
Switch Time	5±3.64	6.74±4.09	9.15±3.21	10.9±2.94
Switch Errors	8.15±3.08	9.29±2.72	9.64±2.97	11.36±1.29
RAVLT				
Recognition	10.85±2.85	11.9±2.9	12.71±2.81	13.82±1.56
Delayed Recall	5.75±2.67	7.62±3.34	9.52±3.45	10.72±2.81
COWAT	36.05±12.82	55.93±15.08	66.97±14.98	73.2±14.31
DSF	7.2±1.11	8.39±1.79	9.52±2.27	11.23±2.65
DSB	3.55±1	4.59±1.72	5.45±2.08	6.64±2.42

Note. D-KEFS Trail = Delis Kaplan Executive Function System Trail Making Test: Switch Time = Switch Time= Number-Letter Switching completion time; Switch Errors = Number Letter Switching errors; RAVLT= Ray Auditory Verbal Learning Test; COWAT= Controlled Oral Word Association; DSF= Digit Span Forwards; DSB= Digit Span Backwards.

Table T3

Descriptive statistics of cognitive scores for males and females

Measure	Males (*n*= 42)	Females (*n*= 118)
	Mean±SD	Mean±SD
D-KEFS TST	4.52 ± 4.16	8 ± 3. 96
RAVLT		
Recognition	11.48±2.45	12.43±2.97
Delayed Recall	6.76± 2.54	8.75±3.66
COWAT	55.89± 15.6	59.8±17.37
DSB	4.51± 1.62	5.03±2.05

Note. D-KEFS TST = Delis Kaplan Executive Function System Trail Making Test: Number-Letter Switching completion time; RAVLT= Ray Auditory Verbal Learning Test; COWAT= Controlled Oral Word Association; DSB= Digit Span Backwards.

Appendix U

Clinical and Demographic Characteristics for Mood Samples

Table U

Clinical and Demographic characteristics for mood samples across the three treatment groups

Clinical and Demographic Characteristics		BAI				BDI				HRV		
	GR	FR	Control	TS	GR	FR	Control	TS	GR	FR	Control	TS
Number of subjects	65	60	67	192	66	61	71	198	41	39	52	132
Age, Mean ± SD (range)	45±10.35 (30-65)	48± 10.56(30-71)	46±9.89 (30-73)	46±10.26 (30-73)	45±10.40 (30-65)	48±10.52 (30-73)	46±10 (30-71)	46 ±10.27 (30-73)	44±10.06 (30-63)	45±10.7(30-73)	44±9.32(30-63)	44±9.91(30-73)
Sex *n(%)*												
Females	47 (72.3)	48 (80)	44 (65.67)	139 (72.40)	47 (71.21)	48 (78.69)	49 (69.01)	144 (72.73)	31 (75.6)	29 (74.36)	33 (63.46)	93 (70.45)
Males	18 (27.69)	12 (20)	23 (34.33)	53(27.60)	19 (28.78)	13 (21.31)	22 (30.99)	54 (27.27)	10 (24.39)	10 (25.64)	19 (36.53)	39 (29.54)
HLE, Mean ±SD	2.32±0.69	2.29±0.55	2.15±0.68	2.26±0.61	2.30± 0.7	2.15 ± 0.68	2.27± 0.69	2.24 ±0.65	2.41±0.78	2.15±0.5	2.29±0.62	2.29±0.7
BMI, Mean (range)	31.36 (18.85-59.31)	29.76 (18.02-47.8)	30.93 (18.82-51.93)	30.72 (18.02-59.93)	31.27 (18.85-59.31)	29.23 (18.02-47.8)	31.09 (18.82-51.93)	30.63 (18.02-59.31)	29.93 (18.76-49.46)	28.70 (17.45-39.45)	30.73 (15.89-49.03)	29.88 (15.89-49.46)
Cholesterol *Mean (range)	5.39 (4.16-7.72)	5.26(4.11-7.67)	5.4(4.01-7.73)	5.02(4.01-7.73)	5.34(4.11-7.72)	5.27(4.11-7.67)	5.41(4.01-7.73)	5.34 (4.01-7.73)	5.34(3.98-7.49)	5.46(4.37-7.55)	5.324(3.88-8.2)	5.37 (3.89-8.20)
High, n	2	3	2	7	1	3	3	5	1	2	2	5
Low, n	2	4	2	8	1	3	4	8	0	1	5	6

Note. Cholesterol and BMI were used as controls for cardiovascular disease risk in the analysis.

*Excluding High and Low.

Appendix V

3 x 2 Factorial ANOVA Outputs for Mood Measures

Table V

Type III ANOVA results using mood measures as criterion

Criterion	Predictor	Sum of Squares	*df*	*Mean Square*	*F*	*p*	η^2	$_{\text{partial}}\ \eta^2$
BAI	Group	53.06	2	26.53	0.93	0.396	0.007	0.008
	Session	**305.02**	**1**	**305.02**	**10.72**	**0.001****	**0.038**	**0.042**
	HLE	13.86	3	4.62	1.16	0.921	0.002	0.002
	Sex	**149.98**	**1**	**149.98**	**5.27**	**0.023***	**0.019**	**0.021**
	BMI	0.04	1	0.04	0.00	0.969	0.000	0.000
	Chol	50.32	1	50.32	1.77	0.186	0.006	0.007
	Age	**388.02**	**1**	**388.02**	**13.64**	**0.000*****	**0.049**	**0.053**
	Group: Session	56.68	2	28.34	0.996	0.371	0.007	0.008
BDI	Group	26.89	2	13.43	0.63	0.534	0.005	0.006
	Session	**130.13**	**1**	**130.13**	**6.09**	**0.015***	**0.024**	**0.027**
	HLE	119.76	3	39.92	1.87	0.137	0.022	0.025
	Sex	**67.09**	**1**	**67.09**	**3.14**	**0.078 .**	**0.012**	**0.014**
	BMI	34.51	1	34.51	1.62	0.206	0.006	0.007
	Chol	**108.38**	**1**	**108.38**	**5.08**	**0.026***	**0.02**	**0.023**
	Age	**242.36**	**1**	**242.36**	**11.35**	**0.01***	**0.045**	**0.05**
	Group: Session	73.81	2	36.91	1.73	0.181	0.014	0.016
HRV	Group	26.97	2	13.48	0.24	0.783	0.003	0.003
	Session	30.88	1	30.88	0.56	0.455	0.003	0.004
	HLE	251.43	3	83.81	1.52	0.213	0.028	0.030
	Sex	62.68	1	62.68	1.14	0.288	0.007	0.008

Criterion

Predictor	Sum of Squares	*df*	*Mean Square*	*F*	*p*	η^2	$_{partial}\eta^2$
BMI	33.48	1	33.48	0.61	0.437	0.004	0.004
Chol	1.64	1	1.64	0.03	0.863	0.000	0.000
Age	**226.30**	**1**	**226.30**	**4.11**	**0.045***	**0.025**	**0.027**
Group: Session	101.41	2	50.71	0.92	0.401	0.011	0.012

Note. BAI = Beck's Anxiety Inventory, BDI= Beck's Depression Inventory, HRV= Heart Rate Variability

Group = Intervention group: Fermented Rooibos, Green Rooibos, and Placebo

Session = Time of measurement: Baseline and Follow-up

HLE = Highest Education level; BMI= Body Max Index.

Cholesterol and BMI were used as controls for cardiovascular disease risk in the analysis.

η^2 = Eta Squared
$_{partial}\eta^2$ was used as measure of effect sizes
*p<0.01. ** p<0.001. *** p<0.000. '.' $p<0.05$

Appendix W

Levels of Depression, Anxiety, and Stress across Sex and Age

Table W

Descriptive statistics for levels of depression, anxiety, and stress across sex and age

Measure	BAI		BDI		HRV	
	N	Mean ±SD	*N*	Mean ±SD	*N*	Mean ±SD
Age						
30-39 years	60	8.42±8.03	63	7.25±7.04	51	54.97±9.04
40-49 years	52	4.7±5.2	54	6.48±6.65	38	51.76±2.29
50-59 years	58	5.34±5.94	57	5.65±6.21	33	49.36±9.48
60-73 years	22	4.59±6.01	24	4.42±3.77	10	55.7±12.19
Sex						
Females	139	6.69±6.87	144	6.74±6.62	93	53.03±9.72
Males	53	4.31±5.85	54	4.89±5.68	39	51.92±9.79

Note: BAI = Beck's Anxiety Inventory; BDI= Beck's Depression Inventory; HRV= Heart Rate Variability