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ORAL SUPPLEMENTATION OF CISSUS POLPUNEA AND CITRUS AURANTIFOLIA ENHANCED SERUM BIOCHEMISTRY AND OXIDATIVE STATUS OF TROPICAL RABBITS

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ABSTRACT

The effect of Cissus populnea stem bark extract (CpSTe), with or without the addition of Citrus aurantifolia extract (CAe), was evaluated on the serum biochemistry and oxidative status of rabbits in the tropics over 34 days. Twenty-four (24) New Zealand White female rabbits were divided into four groups of six rabbits each, designated as Treatment 1-4 (T1-T4). T1 received no extract (Control), T2 was given 2 mL of CAe per kg body weight, T3 received 2 mL of CpSTe per kg body weight, and T4 was given a combination of CAe (1 mL) + CpSTe (1 mL)/kg body weight. At the end of the experiment, a 5 mL blood sample was collected from the marginal ear vein of three rabbits per treatment using a syringe and needle; out of which 3 mL was dropped into plain bottles to obtain serum for biochemical analysis, while a 2 mL blood sample was dropped into heparinized bottles for hematological assessments The proximate and phytochemical analyses of CPST revealed carbohydrate (67.85%) and tannin (126.60 mg/100 g) as the predominant constituents. Administration of CA significantly ($p<0.05$) increased the total serum cholesterol in T2, while a decrease was observed in rabbits under T1 (no administration) and T4. Administering 2 mL of CP (T3) significantly ($p<0.05$) raised the levels of superoxide dismutase (SOD), catalase (CAT), and GSH in the rabbits. The use of CpSTe and CAe separately, rather than combined, could effectively improve the redox status in tropical rabbits.

Keywords: Tropical Rabbits, Citrus aurantifolia, Cissus polpunea, redox status, catalase

INTRODUCTION

Bioactive compounds present in herbal plants have been previously exploited for a host of treatments in conventional medical practice. Thus, the use of herbal plants is as old as mankind itself (Rojas et al., 2022). The use of various ethnobotanicals for therapeutic purposes in humans and livestock is becoming increasingly popular and acceptable globally (Mekonnen et al., 2022). Herbals' magnified acceptability and admissibility could be credited to their supposed safety, availability, and inexpensiveness. The effectiveness of these herbal plants might also be attributed to their innate phytochemical constituents, essential oils, secondary metabolites, vitamins, minerals, and amino acids. *Cissus populnea* is a plant with a wide range of uses in ethnomedicine and food condiment. It is commonly called Okoho or Afato from the family Vitaceae (Ampelidaceae). Apart from being used as a soup condiment, the stem is used to rectify erectile dysfunction and infertility among men in southwest Nigeria (Olooto et al., 2022). According to Akinjiyan et al. (2025), CpSTe extracts cause a feedback inhibition in the expression of drugs targeting the phosphodiesterase 5 gene, while amplifying the expressions of androgen receptor and Nitric oxide synthase genes in erectile dysfunctional rats in comparison to the control group. *Citrus aurantifolia,* on the other hand, has been documented to act as an immune booster, an antimicrobial agent, a strong antioxidant, an anti-cancer, an anti-inflammatory, and it prevents kidney stones. Ugwuoke et al. (2022) reported a dose-dependent elevated body weight, reduced *E. tenella* oocyst production rate, and significantly ($P<0.05$) increased packed cell volume (PCV) of infected broilers after investigating oral administration of different doses of *Citrus aurantifolia* fruit juice (20, 10, and 5 mL/kg body weight) on *Eimeria tenella*-induced coccidiosis disorder in chickens. In another study by Paniagua et al. (2022), a reduction in the concentrate consumption was observed and an improved welfare performance in Holstein bulls given *Citrus aurantium* flavonoid extract supplement was reported.

Herbal supplements in rabbit management are beneficial under extreme climatic conditions. Liang et al. (2022) reiterated that rabbit meat possesses a unique tenderness, lean, flavor, and good sensory properties. Rabbit meat is rich in protein, contains unsaturated fatty acids, conjugated linoleic acid, and minerals, which are easily digested. It also comprises selenium, antioxidant vitamins, and polyamines. Tropical temperature has been documented to range between 21^{0}C – 30^{0}C, while the rabbit's thermo neutral zone/ temperature is between 15^{0}C -21^{0}C. Tropical climate depresses weight gain, reproductive performance, hormonal balance, total protein, albumin, total cholesterol, triglycerides, and oxidative enzyme values in rabbits reared in the tropics (Ebeid et al., 2023). The blood serum biochemical index significantly reflects metabolic changes and organ damage in rabbits. Serum biochemical assay of livestock determines the physiological disposition of animals to nutrition (Sammad et al., 2020). Single use of ethnobotanicals has been documented to be highly beneficial to livestock husbandry and management, expressing phytochemicals ‘potential in alleviating oxidative stress and other medical ailments (Gupta *et al.,* 2006). However, a deep interest in multi-herbal extracts has recently spurred (Herpin *et al.,* 2004; Alem, 2024). This has led the authors of this study to investigate the combined effect of CpSTe and CAe on the serum biochemical indices of rabbits reared under a tropical environment.

MATERIALS AND METHODS

Experimental Site

This experiment was conducted at Mount Olive Farm behind Kwara State University Contact office, Sango, Ilorin, Kwara State, Nigeria.

Source of plant material and extract preparation

CpSTe and CAe were used in this research. Fresh samples of *C. populnea* stem bark and *C. aurantifolia* fruit were obtained from a local herbal practitioner in Ilorin, Kwara State, Nigeria. The *Cissus populnea* stem bark was cleaned with distilled water to remove sand and impurities, then chopped into small pieces. The weighed samples were steeped in sterile distilled water at a concentration of 23 g/100 ml for 72 hours with constant stirring on a mechanical shaker at 250 revolutions per minute. The extract was then filtered. The *Citrus aurantifolia* fruit was also cleaned with distilled water to remove sand and impurities. They were sliced on two sides, steeped at 23 g/100 ml for 72 hours on a mechanical shaker at 250 rpm, and the extract was filtered. The mixture was placed on an orbital shaker (Dlab, China) for 72 hours at 250 rpm for homogenization. The supernatant was sieved with muslin and filtered using Dr Watts' filter paper. All equipment was washed, cleaned, and sterilized.

Experimental Animals and Management

Twenty-four (24) pure New Zealand White rabbits, with an average weight of 1.2 ± 0.4 kg, were used for this experiment. The rabbits were housed at the Animal House at Mount Olive Farm behind Kwara State University, contact office, Ilorin, Kwara State, Nigeria. They were housed in standard, well-ventilated cages with continuous access to food and water. Daily sanitation and standard husbandry practices were strictly followed throughout the experimental period.

Experimental design

The experimental rabbits were randomly grouped in a completely randomized design (CRD) with four treatments and four replicates as follows:

T1 = no administration of extract (control)

T2 = 2 mls of *Citrus aurantifolia* extract (CAe) /kg body weight

T3 = 2 mls of *Cissus populnea* stem bark (CpSTe) extract /kg body weight

T4 = 1mls of *Citrus aurantifolia* extract + 1mls of *Cissus populnea* stem bark extract / kg body weight (CpSTe + CAe)

Data collection

Meteorological Data

Temperature and Relative Humidity

The core body temperature was determined using a digital thermometer, while the pen temperature and relative humidity were determined using a digital hygrometer

Environmental Temperature and Relative Humidity

Ambient temperature (^{O}C) was recorded daily inside the rabbitry using a digital thermo-hygrometer (El-Desoky *et al.*, 2021).

Temperature Relative Index

The overall mean of the maximum and minimum temperatures, RH (%), and the temperature–humidity index (THI) during the experimental period was estimated. The temperature–humidity index (THI) was calculated according to the following equation:

THI = t °C - [0.31 - 0.31RH) (t °C - 14.4),

Where T^{O}C is dry bulb temperature in degrees Celsius, and RH is relative humidity percentage/100. The values obtained are then classified as < 27.85, interpreted as absence of heat stress, 27.8–28.95, moderate heat stress, 28.9–30.05, severe heat stress, and 30.0 and more, very severe heat stress (Marai *et al.*, 2002).

Blood Collection

At the end of 34-day of administration, a 5 mL blood sample was collected from the marginal ear vein of rabbits, when comfortably restrained prior to the blood collection in the cool period of the morning. The blood samples were collected in heparinized tubes, temporarily stored in crushed ice, and transported to a reputable laboratory for analysis.

Results

The proximate composition (%) of the *Cissus populnea* stem bark (CpST) is shown in Table 1. Observation revealed that the CP dry matter value of 93.75%, carbohydrate (CHO) value was 7.85, ash 18.75, crude fiber 11.96, crude protein 4.09 was recorded, and ether extract had the least value 2.35%.

Table 1: Proximate composition of the Cissus populnea stem bark

Parameters	Composition %
Crude Protein	4.09
Ash	18.75
Ether Extract	2.35
Crude Fibre	11.96
Dry Matter	93.75
Carbohydrate	67.85

CP: Crude protein; ASH: Ash; EE: Ether extract; CF: Crude fiber; DM: Dry matter; CHO: Carbohydrate

The phytochemical study of CP evaluated in mg/100g is shown in Table 2. It was revealed that Cissus populnea stem bark contains several bioactive components including alkaloid, terpenoid, saponin, tannin, flavonoid and anthraquinone. After laboratory analysis, the values recorded for each component include 2.14 ± 0.65, 69.14 ± 4.34, 45.28 ± 4.17, 126.68 ± 8.54, 85.45 ± 5.86, and 31.44 ± 6.79 respectively.

Table 2: Phytochemical composition of Cissus populnea stem bark

Parameters	Composition (mg/100g)	SEM
Alkaloid	2.14±0.65	0.26
Terpenoid	69.14±4.34	1.77
Saponin	45.28±4.17	1.7
Tannin	126.68±8.54	3.49
Flavonoid	85.45±5.86	2.39
Anthraquinone	31.44±6.79	2.77

The serum biochemical parameters of rabbits given oral administration of CpSTe and CAe

Table 3 shows the serum biochemical parameters of rabbits given oral administration of CpSTe and CAe. The serum biochemical parameters evaluated include total protein, (TP) high density lipoprotein (HDLC), globulin, albumin, total cholesterol (TCHOL), triglyceride (TRIG), low density lipoprotein (LDL). Among all parameters evaluated, only TCHOL and LDL were significantly ($p<0.05$) affected by the oral administration. It was observed that oral administration of (2ml CA/kg BW) enhanced a significant($p<0.05$) increase in TCHOL and LDL, while the combined administration of CA (1ml) + CP (1ml) caused a significant ($p<0.05$) reduction of TCHOL with a statistical similarity to the control value. An inconsistent numerical increase was also noted across other parameters evaluated. 2 mls CA/ kg BW resulted in numerical increase in the albumin and triglyceride values, while combined effect of CA (1ml) + CP (1ml) numerically improved the Total protein and globulin values.

Table 3: The serum biochemical parameters of rabbits given oral administration of *Cissus populnea* and *Citrus aurantifolia*

Parameter	T1 (Control)	T2 (2ml CA/kgBW)	T3 (2ml CP/kgBW)	T4 (1ml+1ml CACP)	SEM
TP (g/dl)	50.26	51.95	51.77	52.02	0.27

HDLC (mg/dL)	16.11	22.58	30.65	28.78	2.74
Globulin (g/dl)	22.35	21.12	21.89	22.22	2.93
ALB (g/dl)	27.91	30.83	29.88	29.8	2.40
TCHOL (mg/dL)	111.21[b]	167.47[a]	121.50[ab]	108.60[b]	9.73
TRIG (mg/dL)	32.39	46.55	37.34	44.52	2.92
LDL (mg/dL)	88.62[b]	135.58[a]	98.32[ab]	70.92[b]	6.07

CA= Citrus aurantifolia, CIP = Cissus populnea, CACIP = Citrus aurantifolia and Cissus populnea, TP = Total protein, ALB = Albumin, TCHOL Total cholesterol, TRIG Triglycerides, HDLC = high density lipoprotein, SEM = Standard error of means. NS = No significant difference; S = Significant different, T1 = no administration of extract (control), T2 = 2mls of Citrus aurantifolia extract/kg body weight, T3 = 2 mls of Cissus populnea /kg body weight, T4 = 1mls of Citrus aurantifolia + 1mls of Cissus populnea / kg body weight

Oxidative Status of Rabbits given oral administration of Cissus populnea (CP) and Citrus aurantifolia (CA) under tropical conditions

Table 4 shows the oxidative status of rabbits given oral administration of Cissus populnea (CP) and Citrus aurantifolia (CA) under tropical conditions. The significantly ($p<0.05$) highest mean values of superoxide dismutase, (SOD) Catalase (CAT) and Glutathione S-transferases (GST) were observed in T3 (348.43 u/mg, 339.38 u/mg and 7.41 u/mole) with GST showing the numerically highest value (7.41 n/mole). Although T4 shared in the high significant ($p<0.05$) value expressed for Ca (342.2 u/mg), however, rabbits under T4 revealed a significantly ($p<0.05$) high SOD value and numerically lowest GST (5,07 n/mole).

Table 4: The oxidative status parameters of rabbits given oral administration of CpSTe and Cae

Parameter	T1 Control	T2 2mlCAe	T3 2mlCpSTe	T4 1mlCAe+1mlCpSTe	SEM
SOD(u/mg)	296.88[b]	325.42[a]	348.43[a]	278.37[b]	14.62
CAT(u/mg)	292.44[b]	286.34[b]	339.38[a]	342.2[a]	13.94
GST(n/mole)	6.55	6.25	7.41	5.07	0.53

CAe= Citrus aurantifolia extract, CpSTe = Cissus populnea stembark extract, CpSTe and CAe = Citrus aurantifolia and Cissus populnea, SEM = Standard error of means. NS = No significant difference; S = Significant difference, SOD= Superoxide dismutase, CAT= Catalase, GST = Glutathione S-transferases. T1 = no administration of extract (control), T2 = 2mls of Citrus aurantifolia extract/kg body weight, T3 = 2 mls of Cissus populnea /kg body weight, T4 = 1mls of Citrus aurantifoli extract + 1mls of Cissus populnea stem bark extract / kg body weight.

DISCUSSION

The proximate composition (%) of the Cissus populnea stem bark extract (CpSTe), showing 93.75% as dry matter value, mirrored a high concentration of organic compounds and the decreased moisture content depicts a more enduring and nutrient-rich CpSTe powder used for the experiment. Adebowale et al. (2013) reported a carbohydrate component ranging between 43.7 ± 2.5 and 48.1 ± 3.5 g/100 g after studying the proximate composition of CpST flour, the higher value in CHO values recorded in this study, might be due to the use of only dried bark, resulting in variation existing in their chemical composition, as the bark routinely contain higher concentrations of chemical components such as tannins and flavonoids. Stems barks comprise of a higher concentration of carbohydrates (CHO) and proteins, giving rise to a rich energy source as indicated in the CHO value of CpSTe (67.85%). This is suggestive of the potential of CpST to be used as a partial replacement high energy sourced feed ingredient been competed for by man. The percentage ash recorded in this study is higher than the value reported by Achikanu and Ani et al. (2020). This could be due to a higher mineral content peculiar to the stem bark used by the authors of this experiment. The crude protein (4.09%) and ether extract (2.35%) values recorded in this study are higher than the values documented by Agbo (2024) as 2.11% and 2.09% respectively, while the crude fiber reported (22.48%) is higher than the observed value in this study (11.96%).This might be due to the topography, soil type, rainfall difference and the plant variety. However, Akwu et al. (2019) reported a higher CF (55.59%). This might be due to the environmental conditions, plant species, and plant genotype, which could have influenced the chemical composition of the stem and thus the fiber and other values. According to Alex-Asaolu et al. (2024), Cissus populnea Guill. & Perr has been detected as a herbal plant used in ancient folklore as a remedy for microbial infections, venereal diseases, and infertility. Phytonutrients such as flavonoids and carotenoids represent a bondless assemblage of active compounds, which offer significant therapeutic properties with the capacity to enhance the immune system, while acting as an antiviral, anticancer, analgesic, antitubercular, and as anticardiovascular in nature (Ishtiyak and Hussain, 2017). It enhances antioxidant defenses and modulates the gut microbiota. Dey et al. (2020) stated that alkaloids possess medicinal properties like antiviral, antifungal, anticancer, antioxidant, and antispasmodic, therefore highly beneficial to animals when in the right quantity. Terpenoids, also known as isoprenoids, act as precursors for steroids in animals, and some possess anti-inflammatory and traditional antibiotic properties, relevant for treating different diseases and infections. Saponins act as immunostimulants that influence rumen fermentation and microbial populations. Animal researchers apply tannins as in-feed antibiotics to improve animal metabolism, microbiota, growth performance, meat quality, and antioxidant status in ruminants in low to moderate quantities. Flavonoids and Anthraquinones, on the other hand, exhibit anti-inflammatory effects under stress in addition to their antioxidant nature. Yu et al. (2020) marked flavonoids as a potent phytochemical that diminishes methane production in ruminants. Results revealed that CpSB consists of a high concentration of isoprenoids, flavonoids, saponins, and tannins. However, the tannin content in the stem bark was the highest, with 126.68 mg/100g, followed by flavonoid (85.45 mg/100g) and isoprenoids (69.14 mg/100g) values. Stem bark aqueous extract has been widely studied both traditionally and scientifically due to its ability to treat various ailments, including reproductive infections. Tannin ameliorates protein utilization, decreases the incidence of bloat, and possesses antiparasitic effects. Flavonoids upgrade animal growth, enhance meat and milk quality, in addition to antioxidant activity. Achikanu and Onuabuchi et al. (2020) recorded CpSTe alkaloid composition as 1.551 ± 0.03 mg/g, flavonoids 0.761±0.10 mg/g, saponins 2.208±0.07 mg/g, which far exceeds the ones recorded in this study. This might be due to the geographical location, age at harvest, and other factors.

All serum biochemical indices evaluated in this study were within the normal ranges for rabbits as stated by Cumali et al. (2012). This indicates that the treatments were not harmful to the experimental goat. The lowest value of the low-density lipoprotein (LDL) observed in T4 could be due to the combined effect of bioactive components in CpSTe and CAe, which might have worked synergistically to lower the harmful cholesterol (LDL), while improving the ‘good’ or beneficial cholesterol. Serum biochemical analysis is usually an expression of either the immune status, pathology, or nutritional quality of diet type administered to the animal at specific period. Serum biochemical references and oxidative status constitute important markers in the diagnosis, prognosis, and treatment of livestock diseases through the myriads of parameters, including blood and serum biochemical indices within animals according to Ul-Rahman et al. (2023). Much research outputs have revealed that herbal supplements affect rabbits' lipid profiles. Sometimes, it improves some lipid parameters or has a depressive effect on others. While Moringa oleifera and Nigella sativa has been documented to reduce cholesterol, LDL, and HDL levels, Luzardo et al. (2021) has reported that ingestion of Citrus species pulp over 104 days (30% of fresh citrus pulp on a dry-matter basis) did not negatively influence animal performance or carcass and meat quality. Boshtam et al. (2013) reiterated that Citrus species decreases LDL, while HDL and triglyceride were not usually been influenced however, free cholesterol transfer to HDL increased. Citrus has been noted to contain 60 flavonoid varieties with strong antioxidants and anti-inflammatory activities. SOD, GSH-PX and CAT act to prevent free radical formation (Gusti et al., 2021). As enzymatic antioxidant, superoxide dismutase is the first line of antioxidant defense, which eliminates superoxide radicals and prevents oxidative damage to proteins and lipids as stated by Zheng et al. (2023). GSH-PX is a selenoenzyme that detoxifies and reduces lipid hydroperoxides, converting them into water or alcohol (Zou et al., 2021). Catalase is a very stable but efficient enzyme that catalyzes the decomposition of countless hydrogen peroxides into water and oxygen, using a single molecule. Higher values of these antioxidant enzymes maintain the redox balance in an animal system (Rasheed, 2024). The highest mean values of GST, CAT, SOD as observed across T3 showed that the activities of these antioxidant enzymes were increased with the administration 2ml CpSTe. The ability of antioxidants to quench the oxidative stress-mediated damage is believed to contribute to their therapeutic potential in preventing or slowing down apoptosis. Several studies have demonstrated that the antioxidant compounds protect cells by neutralizing the excessive free radicals and/or by enhancing their antioxidant defenses (Chandimali et al., 2025). The involvement of oxidative stress in the initiation or progression of cellular degeneration provides the basis for considering antioxidant therapy as a prophylactic treatment for certain diseases. CP contains a wide variety of secondary components with substantial antioxidant activity compared with other parts of the fruits. They are consumed in large quantities worldwide as fresh produce and juice, most often peels. Akomolafe et al. (2013) also documented the oxidative-stress preventive activities possessed by Cissus polpunea, which are detectable by the DPPHscavenging ability, Fe2+-chelating, and its reducing power contained in its aqueous extractible phytochemicals. The increased activities of antioxidant enzymes may be related to active non-enzymatic antioxidative properties of Cissus polpunea, including ascorbic acid, flavonoids, and phenolic compounds, which corroborate with many previous studies (Rudenko et al., 2014). Citrus extracts, such as citrus peel, C. karna, and C. limetta were demonstrated to contain antioxidant bioactivity. The most important defense mechanism of dry lemon phenolic compounds is based on the absorption and neutralization of free radicals (Impellizzeri et al., 2014). However, previous reports have placed more emphasis on the antioxidant capacity of C. limon (Elwan et al., 2019). C. limon also contains several constituents such as 3-carene, imidazole, D-limonene, geraniol, and squalene, as well as other compounds reported as potent antioxidants that can prevent microsomal lipid peroxide and protein degradation of ROS (Aazza et al., 2011). This research found that Cissus populnea used for this study was high in phytochemical composition, which elevated the value of serum biochemical and enzymes responsible for antioxidant activity in rabbits. In conclusion, Citrus has antioxidant and hypolipidemic effects on rabbits, and it is recommended that the use of Cissus populnea and Citrus aurantifolia as an antioxidant at 2ml CpSTe /kg BW and hypolipidemic for rabbits at the level when in combination with CAe at 1ml CpSTe + 1ml CAe /kg BW. More research is recommended to further investigate the efficacy of these herbal supplements either singly or as a combination in other animal species.

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