RESEARCH ARTICLE

Exploring the Antioxidant Potential of *Cissus woodrowii* (Stapf Ex Cooke) Santapau: A Study on Leaves and Stem

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ABSTRACT

Cancer, diabetes mellitus, cardiovascular disease, neurodegenerative illness, inflammatory disease, and many other pathologies have a common denominator: oxidative stress. The situation arises from the overproduction or ineffective quenching of free oxygen and nitrogen species within the cell. Antioxidant activity, nutritional value and traditionally roots were made into a powder and applied to cut wounds where pus had formed, ethnobotanical/traditional use is as an antitumor in Maharashtra. Owing to its ethnomedicinal importance, proper identification with pharmacognostic and phytochemical details and evaluation is vital for drug development and to prevent adulteration highlights and their role in laying down standardization. The present paper discusses phytochemical and antioxidant study of *Cissus woodrowii* (Stapf ex Cooke) Santapau. The both extract showed occurrence of glycosides, phenolic compounds, alkaloids, flavonoids also tannin and aqueous and ethanol extract of stem and leaves showed good antioxidant properties by DPPH assay.

Keywords: *Cissus woodrowii* (Stapf ex Cooke), Pharmacognostic, Phytochemical, Antitumor, Antioxidant.

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INTRODUCTION

In addition to being produced endogenously during the body's regular aerobic metabolic activities, nitrogen and free oxygen species are also found in the environment (exogenous).[1,2] Free radicals can also be produced by exposure to exogenous factors including smoking, ionizing radiation and medications. In contrast, free radicals are produced endogenously by mechanisms like the mitochondrial electron transfer chain as well as by various diseases.[3] There is an intricate antioxidant defense mechanism in the body that includes both enzymatic and non-enzymatic pathways that ensure health by keeping pro-oxidants and antioxidants in a state of dynamic balance.[1] Three enzymes catalase, glutathione peroxidase, and superoxide dismutase comprise the enzymatic antioxidants. Antioxidants that are not enzymes are also used by the body; examples include uric acid, bilirubin, and lactoferrin. However, oxidative stress is linked to many diseases, and this damage is caused in part by the accumulating free radicals when the body's endogenous antioxidant mechanisms are overworked.[4]

To combat oxidative stress, scientists have conventionally used synthetic antioxidants. There are reports of negative side effects from using these synthetic antioxidant molecules.[5] Examples include the carcinogenic and hepatotoxic effects of butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Synthetic antioxidants are underutilized because they are difficult to obtain, expensive, and easily damaged.[3] Current work is necessary to identify replacement antioxidants that are more secure, conveniently accessible, and potent.[6] Medicinal herbs have a higher likelihood of offering effective, safer, less expensive, and more simply reachable remedies for oxidative stress-related ailments than current conventional and complementary approaches.[7] Plants' antioxidant qualities have been shown to protect the body from disease, with studies showing that their eating reduces chances of cancer, cardio disease, dementia, high blood pressure, and stroke.[8]

Polyphenols and three B vitamins (C, A, and E) are the main classes of phytoconstituents responsible for plants' antioxidant activity. The hydroxylated versions of benzoic acid and cinnamic acid found in plants are called phenolic compounds. They can fight cancer and free radicals.[3] To name a few, there are tannins, anthocyanidins, coumarins, flavonoids, and phenols. The defense systems of plants against

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biotic and abiotic stressors rely heavily on these phytoactive compounds.[9] Ingesting plant products or plants high in these phytoconstituents components is thought to have similar health benefits for humans.[8] Example, flavonoids have been known for properties that fight inflammation, allergies, viruses, immune system issues, aging, and cell growth. In a healthy body, these substances keep the balance between prooxidants and antioxidants, which is good for health.[1] There are three enzymes that act as antioxidants: glutathione peroxidase, catalase, and superoxide dismutase. On the other hand, the body also uses nonenzymatic antioxidants such as bilirubin, uric acid, and lactoferrin. However, oxidative stress is linked to many diseases, and this damage is caused in part by the accrual of free radicals when the body's endogenous antioxidant mechanisms are overworked.[4] Various synthetic antioxidant chemicals, including BHA, BHT and PG, are typically used to control oxidative stress. These synthetic antioxidant molecules are used; however, they have some unintended consequences.[5] For illustration, BHT and BHA have been shown to be cancer-causing and to produce hepatotoxicity. Synthetic antioxidants are underutilized because they are difficult to obtain, expensive, and easily damaged.[3] Hence, the present research is necessary to address the demand for alternative antioxidants that are not only potent but also safely accessible, given the severe repercussions.[6] The potential of medicinal herbs to provide efficacious, risk-free, cost-effective, and readily available remedies for maladies associated with oxidative stress surpasses that of contemporary conventional and complementary methodologies.[7] Consumption of plants with high levels of antioxidants has been demonstrated to defend the body from ailments, with benefits including lower rates of carcinoma, coronary artery disease, hypertension, dementia, and stroke.[8]

Plants' antioxidant activity is due in large part to the presence of phytochemicals.[3] To name a few, there are tannins, anthocyanidins, coumarins, flavonoids, and phenols. The defense systems of plants against biotic and abiotic stressors rely heavily on these phytoactive compounds.[9] Ingesting plants or plant products high in these phytoactive components is thought to have similar health benefits for humans.[8] Flavonoids, for example, have long been known to provide a variety of health benefits, including those related to inflammation, allergies, viruses, immune modulation, slowed aging, and cell proliferation.[10]

Research on natural antioxidants, such as those found in food and medicine, has increased dramatically as people look for safer alternatives to synthetic antioxidants.[3,10] The Asteraceae family includes the shrub *Vernonia lasiopus* (O. Hoffman.). Traditional herbalists in Kenya utilize *V. lasiopus* leaf infusions and decoctions to cure malaria, inflammation, epilepsy, pain, and other conditions.[11,12] Antioxidant properties have been previously ascribed to several members of the Asteraceae family of plants. For example, Iwalokun *et al.* found that an aqueous solution of *V. amygdalina* leaves could protect mice from hepatotoxicity and oxidative stress caused by acetaminophen.[13]

MATERIALS AND METHOD

Plant Materials and Processing

Cissus woodrowii (Stapf ex Cooke) Santapau stem and leaves (Figure 1) were harvested fresh from their native environments in Kolhapur. We consulted with local herbalists and conducted a thorough ethnomedical survey to choose which plants to use.[12-15] Grape tree *woodrowii's*, girnool, and other medicinal plants were initially discovered with the aid of a trusted local herbalist using just their common names and the diseases they are known to treat. For botanical verification, we provided the samples to a respected taxonomist. Dried plant components (Figure 2) were then ground to powder by an electric plant grinder before being stored in sealed containers and kept at room temperature pending extraction.

Preparation of Ethanolic Extracts

Each plant substance was powdered and placed in a 2 liter conical flask with 1-liter ethanol. Each plant substantial part was placed in soxhelet apparatus and run eight cycles and let to remain at room temperature for 48 hours (Figure 3). The menstruum was sorted in all of them by filtering them by Whattman-1 filter paper. Filtrates were dried in a hot-air oven set to 35°C after being concentrated in a rotary evaporator at 50°C. Prior to their usage *in-vitro* bioassay, concentrates were sealed in airtight vessels and kept at 4°C.[16]

Preparation of Aqueous Extracts

Maceration: The leaves or stem bark are finely pulverized and placed in a container for the extraction process (Figure 4). Once the drug material is covered, the menstruum is put on top of it. After three days have passed, the container can be discarded.[1-4,11,16] To achieve thorough extraction, the contents are mixed at regular intervals and, if placed in a bottle, shaken at regular intervals. Micelle and marc are separated by filtration or decantation. Menstruum is subsequently removed by evaporating the micelle in an oven.[1-4,11,16] Thermosensitive plant material benefits greatly from this strategy.

Figure 1: Fresh stem and leaves *C. woodrowii* (Stapf ex Cooke)

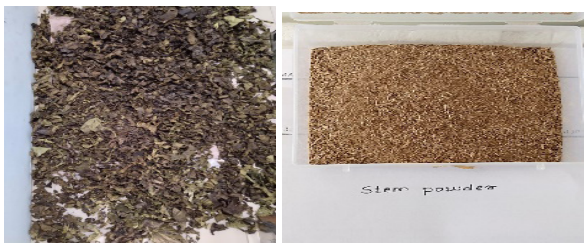

Figure 2: Leaves and stem powder *C. woodrowii* (Stapf ex Cooke)

Figure 3: Preparation of alcoholic extracts stem and leaves of *C. woodrowii* (Stapf ex Cooke)

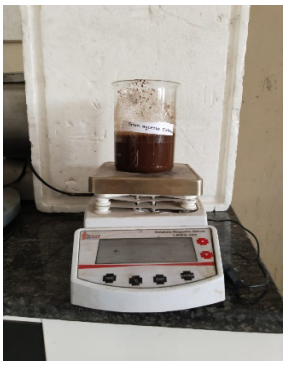

Figure 4: Preparation of aqueous extracts of stem and leaves of *C. woodrowii* (Stapf ex Cooke)

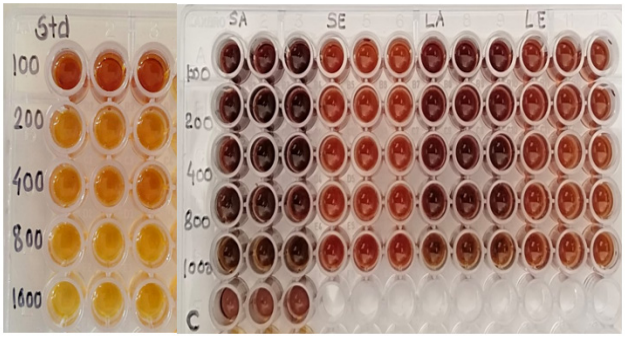

Figure 5: Well plate method for antioxidant action of stem and leaves aqueous, ethanolic extracts of *C. woodrowii* (Stapf ex Cooke) Santapau

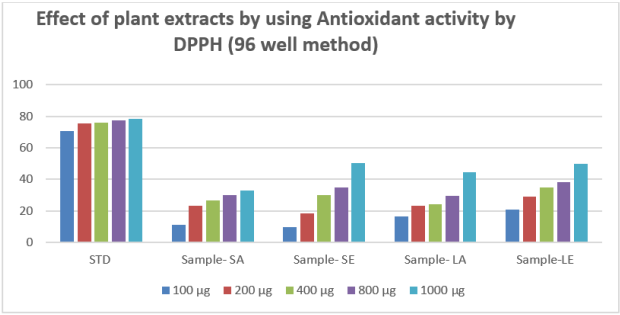

Figure 6: Graphical view of effect of plant extracts by using antioxidant activity by DPPH.

Qualitative Phytochemical Screening

Standard phytochemical screening protocols were used to conduct qualitative assays for several phytochemicals found in the aqueous and ethanolic leaf stem bark extracts of *C. woodrowii* (*Vitis woodrowi*). The presence or absence of a specific class of phytochemicals could be visually determined by inspecting the look of color or foaming.[16]

Test for saponins

It was weighed out that 2 grams of each of the plant extracts being tested could be mixed with 5 mL of distilled water. After that, 2 mL extract solution was agitated for 30 seconds. Preparations were left alone for 15 minutes to settle. Samples containing saponins will continue to foam for more than 15 minutes after being examined.[16]

Test for alkaloid

Every of the investigated plant extracts was combined with 10 mL of 0.1M HCL in a volume of 2 grams. Heated in a water bath at 50°C for 5 minutes. Three drops of Dragendorff's reagent were mixed. The reddish-brown discoloration of the sample specifies the existence of alkaloids.[16]

Examination for terpenoids

Two mL of alcohol extracts and five drops of acetic anhydride were combined in clean test tubes. Carefully working through the test tube's side, 5 drops of sulfuric acid were added afterward. The presence of terpenoids is shown by the creation of a blue ring at contact.[16]

Test for flavonoids

In 5 drops of conc. HCl were added 2 mL of alcohol extracts of plants under study. When a crimson hue develops, flavonoids are present. In 2 mL of the alcoholic extracts had 1-mL of diluted ammonia added to them, and the mixture was stirred slowly. Flavonoids are identifiable by their characteristic greenish yellow.[16]

Test for cardiac glycosides

Two drops of a 10% ferric chloride solution were added to 2 mL of glacial acetic acid, and then 0.5 grams of the extract was added to the mixture to test for the presence of cardiac glycosides. After that, we slowly added 1-mL of concentrated H_2SO_4 to the base mixture. If there is a pink band at the edge, it means that the deoxy sugars (cardenolides) are present.[16]

Test for steroids

In this research, steroids' presence in plant extracts was evaluated. In 2 mL of chloroform were mixed with each extract until the mixture was about half a gram in weight. Then we put in three drops of the Lie-Burchard reagent and shook it up a bit. A reddish-purple color means that steroids are present.[16]

Test for phenols

About half a gram of each plant was boiled in five minutes in 5 mL of 70% C_2H_6O in a water bath to make plant extracts. In 5 drops of 5% $FeCl_2$ were mixed after cooling, and the mixture was agitated. Phenols are present in the sample if a green precipitate forms.

Estimation of Total Phenolic Contents

Overall the containment of phenol was calculated by an improved kind of the Folin-Ciocalteu technique, based on research by Do *et al.* To begin, 2 mL of the Folin-Ciocalteu reagent was blended to 1-mL of extract. To create a reagent, it was diluted with distilled water at a 1:10 volume/volume ratio. A subsequent addition of 1-mL of 20% sodium carbonate was made. Blend was incubated at 40°C for 30 minutes before being shaken for 20 seconds. Absorbance was checked at 765 nm. For standard curve, gallic acid was employed. Gallic acid equivalents (GAE) were utilized to measure total phenolic content of the extracts tested. Three separate studies yielded the same results.[17-20]

Estimation of Total Flavonoid Contents

The overall contain of flavonoid in the extracts was measured using a procedure published by Park *et al.* A 10 mL test tube was filled with a solution containing 0.3 mL of extracts, 3.4 mL of 30% CH_3OH, 0.15 mL of 0.5 molar $NaNO_2$, and 0.15 mL of $AlCl_3$ $6H_2O$ (0.3 M). Five minutes later, subsequently mixing in 1-mL of 1 M sodium hydroxide, the absorbance at 510 nm was assessed contrary to a blank. A standard curve showing the relationship between total flavonoid content and quercetin concentration was between 0 to 100 mg/mL. Quercetin mg per mg of sample weight were provided as an indication of total flavonoids. The research was performed three times.[21]

Determination of *In-vitro* 1, 1-Diphenyl-2, Picryl-Hydrazyl) activity

Antioxidant activity was determined by measuring sample compounds' ability to neutralize DPPH free radicals (George *et al*., 1996). Test chemicals and water volume totaled 100 µL in each microtiter plate. In 100 µL of 0.1% methanolic DPPH was put above every specimen as well as subjected to incubation for 30 minutes in dusky conditions. Elisa plate reader at 490 nm was used to check for discoloration in samples; purple, yellow, and pale pink were deliberated robust and weak positives, accordingly. The following formula was utilized to determine radical scavenging action:

DPPH Activity (%) = [(Absorbance of control - Absorbance of test sample)/(Absorbance of control)] x 100 ---- (1)

RESULTS

Qualitative Phytochemical Screening

The plant extracts that were studied had saponins, terpenoids, flavonoids, alkaloids, and phenols found in them through qualitative phytochemical tests. But the leaves and stem of *C. woodrowii* Santapau did not have any heart glycosides or steroids (Table 1).

Antioxidant Activity – DPPH Radical Scavenging Activities of Aqueous and Ethanolic leaves and stem *C. woodrowii* (Stapf ex Cooke) Santapau Extracts

The ethanol solution absorbs light most strongly at a wavelength of about 490 nm, indicating that the deep violet color is caused by the delocalization of electrons. By combining DPPH with a substrate (AH) that can contribute a hydrogen atom, the violet pigment is removed, giving rise to the reduced version. According to the Table 2, the samples at the concentrations 1000, 800, 400, 200,100 µg/mL. Sample-Stem ethanolic (SE) and sample- Leaves ethanolic (LE) and Sample- Stem ethanolic (SE) showed good activity as compared to standard (Figures 5 and 6).

Table 1: Qualitative phytochemical composition of aqueous and ethanolic leaves and stem *C. woodrowii* (Stapf ex Cooke) Santapau extracts

Phytochemicals	*SE (Stem Ethanolic)*	*SA (Stem Aqueous)*	*LE(Leaves Ethanolic)*	*LA(Leaves Aqueous)*
Steroids	−	−	+	−
Phenols	+	+	+	+
Alkaloids	+	+	+	+
Cardiac glycosides	-	-	+	-
Saponins	+	+	+	+
Flavonoids	+	+	+	+
Terpenoids	+	+	+	+

+, present; −, absent

Table 2: *In-vitro* DPPH scavenging actions of aqueous ethanolic extracts of aqueous and ethanolic leaves and stem *C. woodrowii* (Stapf ex Cooke) Santapau extracts.

Concentration in 1-µg/mL	*L-ascorbic acid*	*SE*	*SA*	*LE*	*LA*
100	70.74	11.04	9.41	20.93	16.58
200	75.61	23.33	18.31	29.93	23.06
400	76.19	26.47	30.14	35.00	24.22
800	77.18	29.87	34.74	37.99	29.35
1000	78.54	32.70	50.44	50.02	44.63

DISCUSSION

Reactive oxygen and nitrogen species are being made more and antioxidant mechanisms in the body are not working as well. This leads to oxidative stress.[22,23] Aerobic organisms and healthy cells both produce ROS/RNS, although at carefully regulated rates.[24] Increased considerably, leading to subsequent changes in proteins, lipids, and nucleic acids in membranes.[6] Atherosclerosis, cancer, ischemia-reperfusion injury, and neurodegenerative diseases are just some of the pathogenic processes linked to oxidative degradation of these biomolecules as we age.[25] Complex antioxidant mechanisms have evolved in humans to protect body from ROS and RNS and to keep the redox system in balance.[26] There are both endogenous and exogenous antioxidant defense systems in the body.[27-30]

Hepatic damage and cancer have been linked to synthetic antioxidants currently engaged in the direction of battling oxidative stress; they include PG, BHA, and BHT. They also show weak efficacy in both animal and human studies.[31] Antioxidants found in plants are increasingly being considered as a viable alternative to synthetic antioxidants due to their lower toxicity, greater availability, and lower cost.[32,33]

Antioxidant activities, according to the literature, should not be determined using only an antioxidant experimental model.[6] Anti-oxidant activity is often evaluated using one of

several *in-vitro* testing methods.[6,34] ORAC testing FRAP, nitric oxide scavenging assay, and DPPH scavenging effects are all examples of *in-vitro* antioxidant tests that are employed.[34,35]

In this study, we used a ferric reducing antioxidant power assay.[17] This method relies on the analyte's capability to reduce ferric (Fe3+) ions to ferrous (Fe2+) ions.[35,36] Therefore, 700 nm absorbance capacities can be used to analyze Fe2+ formation. When absorbance at this wavelength rises, reducing power also rises.[36]

Methanolic extracts of *C. volkensii*, *V. lasiopus*, and A. hockii were found to have strong ferric-reducing antioxidant capacity, as showed by a rise in absorbance values as a function of concentration. Results were consistent with an *in-vitro* investigation showing that Bauhinia rufescens Lam leaf extracts have antioxidant properties by Aliyu *et al.*[15] In addition, Adesanoye and Farombi discovered that *Vernonia amygdalina* methanolic leaf extracts exhibit dose-dependent ferric reducing activity.[37] Furthermore, our consequences are in arrangement with those of Sowndhararajan *et al.* demonstrated the antioxidant activities of methanolic preserved extracts of the bark of Indian Acacia classes.[38] In addition, the plant extracts were tested for their antioxidant abilities according to standards set forth by Do *et al.*[20] All of the plant extracts tested had EC50 values <50 g/mL; hence they were all regarded to be potent antioxidants. The study's findings backed up those of Fidrianny *et al.*, who found the same things in *Momordica charantia*.[22] Antioxidant efficacy has traditionally been measured using the DPPH radical scavenging technique.[18] By this method, antioxidant properties are assigned to compounds based on their capability to quench DPPH free radicals *in-vitro*. A shift in color from blue to yellow, measured in nanometers (517 nm), is indicative of DPPH activity.[18] DPPH effects were graded rendering to criteria of Fidrianny *et al*, and entirely plant extracts tested had IC_{50} values lesser than 50 mg/mL, making them potent antioxidants.[22]

In addition, this study looked into whether or not the plant extracts had the ability to scavenge for hydroxyl radicals. Enzymes in the body are damaged by hydroxyl radicals because they oxidize thiol (-SH) groups, according to studies.[39-42] The Fenton reaction produces the hydroxyl radicals. An antioxidant sample that can scavenge hydroxyl radicals *in-vitro* is thought to have promising benefits *in-vivo* as well.[43]

Similar to what was found in earlier studies on mutually acetone as well as water-based entire extracts of *Bulbine abyssinica*, this study found that concentration of plant extracts reduced hydrogen peroxide's ability to scavenge.[44] On the other hand, some research has found that hydroxyl radical scavenging activities increase with increasing concentration.[45] Higher extract concentrations may have lower actions because of the inundation of reactive centers of hydroxyl radicals, whereas those at more dilute concentrations have high activities because of the ease and quickness of their reactions.[46-50]

CONCLUSION

Results showed that phytochemicals like alkaloids, saponin, flavonoids of the both extracts of stem and leaves showed good antioxidant activity by using DPPH assay by reduction of free radicals which indirectly helps in prevention of cancer, diabetes mellitus, cardiovascular disease, neurodegenerative illness, inflammatory disease. More study is needed to identify pure photoactive components and determine how best to use them.

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