REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 24, No. 4 (2023)
http://www.veterinaria.org
Article Received: Revised: Accepted:

Biosynthesis Of Ethanolic Extract Using Clitoria Ternatea And Estimation Of Antioxidant Capacity

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INTRODUCTION:

As it changes colour toward blue, Clitoria ternatea or butterfly pea's reproductive blossom is admired and valued highly. Like the rest of the family Fabaceae, this plant too has 58 species which are known to occur in a globe shape 1. This Perennial herb is also cultivated in cultural and medicinal aspect in places like Bengal and India. "Blue" tea, renowned internationally, derives its name from the butterfly pea plants sepal and as blue tea's colour also changes in accordance with the acidity of the medium—hypothetically shifts during infusion to alkaline water and citric acid. When citric acid is added to the tea pot, it indeed turns violet which suggests acidity 2.

The listed component holds therapeutic capabilities because of its phytoactive compounds, butterfly pea for instance. The chamomile characterized by blue-purple colour is also known for having extraordinary effects and that is because of its anthocyanin content which has powerful oxidation inhibiting action.

Some fruits that consist of the water-soluble, pH-dependent phenolic compound anthocyanin include blackberries, strawberries, and cherries (3). Since they are capable of neutralising free radicals, anthocyanins will protect the cells from oxidative stress. The healing properties of Clitoria ternatea as well as its ability to escape high metabolism and low energy encompass the traits mentioned above.

Apart from the antioxidant benefits, Clitoria ternatea is known to have multiple applications in Ayurvedic medicine. Using several parts of the plant, it is believed to help with skin disorders, arthritis, and other gastrointestinal disorders (4). Accompanying the several phytochemicals, the medicinal value ot this plant is enhanced. One of the key phytochemicals present in Clitoria ternatea, acetylcholine, has an important role in increasing brain function. It relieves anxiety but increases memory neurotransmission, thus reducing anxiety (5). This is the reason Clitoria ternatea is considered an herbal treatment for dementia and other cognitive disorders. The efficiency of the plant as medicine is also due to the presence of quercetin and kaempferol glycosides.

Besides possessing anti-inflammatory and anti-oxidative properties, Quercetin is also useful in controlling inflammation, blood sugar levels, heart activity, and even the apoptosis of certain types of cancer (6). Kaempferol is known to enhance the body's oxidative stress protective mechanisms, thereby increasing defense against free radicals. Tumor angiogenesis, inflammation, metastasis, and cell apoptosis are some of the many vital processes affected by the development of cancer and that Kaempferol impacts. Increasing the bioavailability of Kaempferol using nanotechnology is thought to significantly enhance its therapeutic benefits (7).

Phyto-clorophyll activism has shown that Clitoria ternatea has a broad range of phytochemicals making it an exciting and potent natural source for anti-oxidants. The comprehensive research that is being carried out about the biological properties of the plant will enhance its importance in both modern and traditional medicine.

An ethanolic extract of Clitoria ternatea phytochemically screened has been evaluated in the present study for its free radical scavenging potential. The goal of the scientific work is to study the effectiveness of the herb on the health promoting role through the stimulation of the antioxidant defences of the organism.

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MATERIALS AND METHOD:

Preparation of Extracts:

Swelling 1 gramme of Clitoria ternatea in ethanol 1:150 solution for a day while stirring provided the extract shown in figure 1. This transformation occurred at room temperature. In order to encourage the extraction of the bioactive constituents, the extract underwent exposure to a water bath set at 40 degrees Celsius for 10 to 15 minutes. Whatman No. 1 filter paper was employed to remove plant residues by filtering. The liquid part of the filtrate was concentrated to 5 mL and subsequently refrigerated along with the rest of the extract for further testing.

Figure 1: Prepared ethanolic extract of C.ternatea

Antioxidant activity:

1. DPPH METHOD

This text covers the workload on antioxidant activity analyses of ethanol extracts of Clitoria ternatea done through DPPH technique which include preparation of extracts and secondary analyses. The expression of activity in this case includes preparation of extracts where each dosage level ranged from 10 µL to 50 µL in increments of five. For each solution set, the concentration of DPPH was kept at 1 mL and at 0.1 mM DPPH while the volume of Tris-HCl used was kept under 50mM in a 5mL volumetric flask. To aid with the extraction concentration process, the 1 mL of the DPPH solution was housed in methanol, over the course of the experiment the samples would be incubated in the dark for half an hour and undergo spectroscope measurementss post the 30 minute mark. It was discovered after the experiment that the inhibition potential of the extract was able to maintain proportionality with the quotient described in the methods section.

% Inhibition = (Absorbance of Control − Absorbance of Test Sample)/Absorbance of Control × 100

This method assesses the Clitoria ternatea extract's antioxidant activity by measuring its ability to neutralise DPPH free radicals.

2. HYDROXYL RADICAL SCAVENGING ASSAY:

We optimized the reaction parameters, then investigated the potential Clitoria Ternatea ethanolic extract scavenging activity using a deoxyribose degradation assay. Each solution was prepared automatically. The reaction mix of 1.0 mL contained 500 microliters of Clitoria ternatea extract at the specified concentrations of 10 microliters, 20 microliters, 30 microliters, 40 microliters, and 50 microliters, 100 microliters of 28 mM 2-deoxy-2-ribose (in phosphate buffer, pH 7.4), 200 microliters of 200 micromolar iron (III) chloride and 1.04 millimolar ethylene diaminetetraacetic acid, vowing to (1:1) in 1:1 (5 mL:5 mL) v/v, 100 microliters of 1 millimolar hydrogen peroxide, and 100 microliters of 1 millimolar ascorbic acid. Incubation at 37 degrees for one hour was required. The measuring wavelength of 532 nm was used for monitoring the deoxyribose cleavage reaction using a blank solution to zero out the baseline. Within this experiment, the positive control used was vitamin E, consistent with several other experiments (9). This methodology has demonstrated selective scavenging of hydroxyl radicals and protection against oxidative subcellular damage by Clitoria Ternatea extract, supporting the hypothesis.

RESULTS

The results from the DPPH and H2O2 assays indicate that the ethnolic extract of Clitoria ternatea possesses antioxidant activity in comparison to the control. The control demonstrated almost 70% inhibition with 10µL of the DPPH test, while the extract achieved just under 60% inhibition with 10µL; however, the extract's inhibitory effect at higher doses was

much greater. The activity of the extract increased in a dose-dependent manner until reaching a plateau at 30µL, where the inhibition matched that of the control, suggesting that greater doses are more effective. This pattern was also observed with the H2O2 test, where the extract displayed mild inhibition at 10µL, identical to the control, but at 40 and 50µL, the extract exhibited much stronger inhibition with approximately 90%, which was considerably greater than the control. Findings from this study strengthen the growing belief that ethnolic extract of Clitoria ternatea is a naturally occurring anti-oxidant substance and confirm the extract's anti-oxidative properties are dose-dependent.

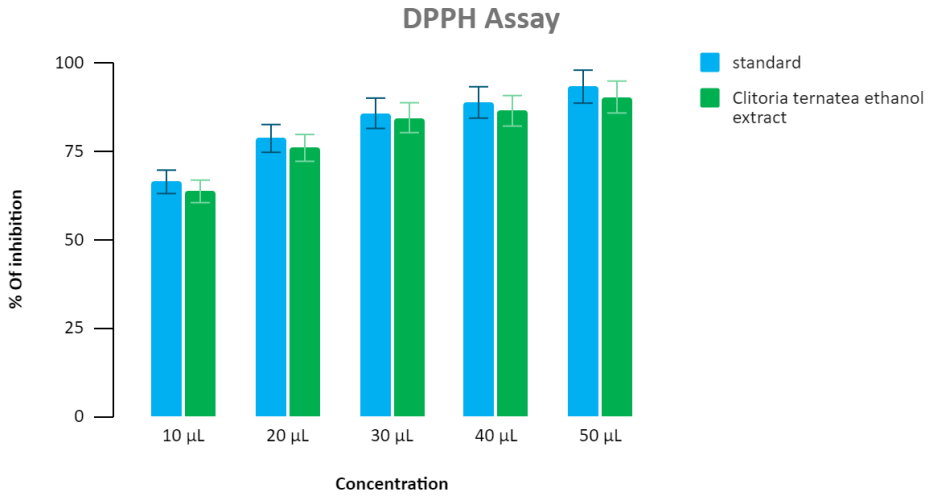

Figure 1: Antioxidant activity of the prepared extract compared with the standard sample that is vitamin C using DPPH assay

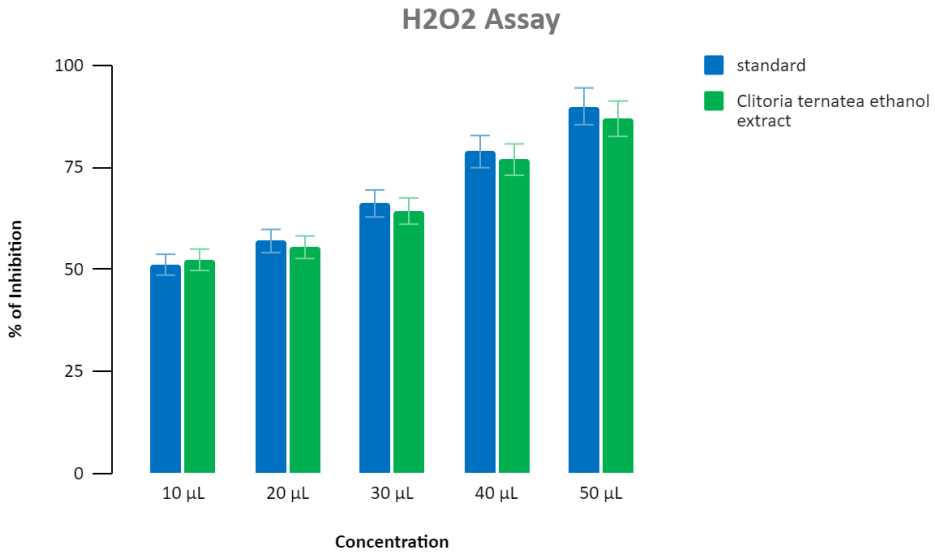

Figure 2: Antioxidant activity of the prepared extract compared with the standard sample that is vitamin C using H2O2 assay

DISCUSSION:

It was demonstrated that antioxidant activity and the concentration of Clitoria ternatea ethanol extract are positively correlated. It has been shown that at higher doses, its antioxidant activity is similar to that of vitamin C which indicates the possibility of the presence of some active bioactive compounds (10). The substantial antioxidant activity is due to anthocyanins which are present and change color depending on pH (11). Phenolic pigments known as anthocyanins are major antioxidants that actively defend cells against the harms of oxidative stress by scavenging free radicals (12).

The cyan blossoms of Clitoria ternatea are an untapped reservoir of antioxidant and glycosides such as quercetin and kaempferol, working synergistically, reinforces the antioxidant potential of the plant (13). Quercetin is one of the most popular phytochemicals due to the association of oxidative stress with necrosis and apoptosis, and this sad obsession with

free radicals (14). Another key phytochemical is the essential anti-inflammatory, anticancer, and cardioprotective phytochemical kaempferol (15). These combinations of phytochemicals increase the antioxidant potential of the plant, illustrating the vast potential for products aimed at oxidative stress.

Consistent with previously conducted studies, Clitoria ternatea was noted to dampen cellular damage resulting from oxidative stress and lipid peroxidation (16). On top of that, the protective effects C. ternatea exerts against oxidative stress aids in retention and cognitive functioning (17). Moreover, the plant's anthocyanins and flavonoids impact on pro-inflammatory cytokines, which indicates that its antioxidant activity may aid in the treatment of inflammatory diseases (18).

The antioxidant properties of C. ternatea suggest the plant's phytochemical profile makes it particularly interesting for further pharmacological studies. However, it must be noted that its antioxidant properties are somewhat weaker than that of ascorbic acid. exploitable targets to complement drug formulation technologies. Considering C. ternatea antioxidant capacity and its potential application in chronic diseases linked to oxidative stress, such as metabolic syndrome, neurodegenerative disorders, and cardiovascular diseases, and stroke makes it a possible candidate for treatment (19). Its bioavailability and therapeutic effectiveness could be increased through research focused on nanotechnology approaches (20).

Conclusion

Our main focus while researching Clitoria ternatea was its sustaining antioxidant and free radical scavenging activities. In addition, the herbs antioxidant activity is largely due to its phytochemical composition especially its anthocyanins, quercetin, and kaempferol glycosides. Although C. ternatea appears to have lesser potency than vitamin C, its presumed antioxidant properties indicate it could be valuable in the formation of pharmacological approaches intended to help manage oxidative stress and other conditions.

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