Haemolytic and Antioxidant Activity of Saponin Glycosides Isolated from Seed Powder of *Cleome viscosa*

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Abstract

Glycosides which are chemicals usually present in the plants and animals have many applications in the biology and medicine. *Cleome viscosa* belonging to the family *Cleomaceae* have significant bioactive molecules with wide applications. Due to the tremendous applications of glycosides and the *Cleome viscosa*. In this study the seeds of *Cleome viscosa* were selected to extract and identify the presence of saponin glycosides and evaluate the haemolytic and antioxidant properties. The *Cleome viscosa* plant were collected from premises of Osmania University, Hyderabad, Telangana, India. The haemolytic activity and antioxidant activity of saponin glycosides extracted by analytical method from *Cleome viscosa* were evaluated. The saponin glycosides extracted from the seeds of *Cleome viscosa* can be used in medicine due to its haemolytic and antioxidant potential.

Keywords: *Cleoma viscosa*, Saponin glycosides, Haemolytic activity, Antioxidant activity.

INTRODUCTION

Saponin glycosides are plant compounds that form soapy foam. when mixed with water. Because of this, they are often used as natural detergents (Aruna Jyothi Kora 2023). When saponins are broken down (hydrolyzed), they produce sugars and non-sugar part called aglycone. The aglycone part, known as sapogenin has a special chemical structure called a spirochetal side - chain, saponins are also used in medicine (Hamdi, A et. al 2024), foaming agents (Badve, M., & Humbare, T 2023), as fire extinguishers in fire incidents and even as fish poisons. Fish are killed when sapogenins are added to water, but they are still safe to eat because saponins are not harmful to humans when taken by mouth.

Saponin glycosides has wide applications in pharmacy and medicine (Man, S. et. al 2010; Onlom, C. et. al 2017; Shastri, V.P & Pino, C. 2008; Kawabata, T. et. al 2011), agriculture, industry (Price, K.R. et. al 1987), cosmetics personal care (Tamura, Y. et. al 2012), veterinary etc., (Table 1).

Plant name	Part of plant	Method of saponin extraction	Applications	Reference
Dodonaea viscosa JACQ	Leaf	Soxhlet and analytical	-	Bajad, P.N. et. al 2019
Lepudium aucheri boiss	Plant	Soxhlet method	Blood hemolytic activity and Acute toxicity	Sultan, M. Q. et. al 2022
Hedera Helix Algeriensis	Leaves	Maceration, Response surface method	-	Nadjia Sabri and Nadji Moulai-Mostefa 2020
Jatropha curcas L.	Leaves and stem bark	Soxhlet extraction method	Surfactants	Rai, S. et. al 2023
Quillaja bark/ Liquorice/ yucca	Bark	Maceration, percolation, decoction modes, Soxhlet extraction	Anticancer activity, hemolytic activity	Timilsena, Y. P. et. al 2023
Camellia sinensis	Flower	High performance liquid chromatography	Anti-cancer	Yaomin, Wang et. al 2017
Sapindus emarginatus	Powered plant material	Maceration	Anti-inflammatory, anti-pyretic	C.R.Shibu Prasanth et. al 2023
Gypsophila simonii	Root	Acid hydrolysis	Anti-inflammatory, anticancer, antimicrobial, anti-invasive effects	A. Nihal YÜCEKUTLU 2016
Cleome viscosa	Root	Analysis, solvent extraction	-	Abdulaziz M 2023

Table 1: Review literature of saponin glycosides extracted from various plant sources and their applications

Cleome viscosa which is also known as wild mustard belong to a family *Cleomaceae.* It is an annual plant found universally and used as a medicinal plant as it has many biological activities (Gupta, et. al 2012). It has a wide variety of pharmacological constituents which have different medicinal actions. The younger plant parts of *Cleome viscosa* are consumed as edible vegetables.

In Ayurveda, it is used as an Anti-helminthic (Chandak, R.R. & Bhairat et.al 2010), pruritic and can be used to treat diseases like gastrointestinal problems (Parimala Devi, B. et. al 2002, Mohtasheem ul Hasan, M. et. al 2011), ringworm, flatulence, colic, dyspepsia, cough, bronchitis, cardiac disorders, inflammation of the middle ear (Wake, Rajesh & Patil et. al 2011), applied to wounds and ulcers (H. Singh et.al 2017), shows hepatoprotective activity (Gupta, N. K., & Dixit, V. K. 2009) and used as rubefacient and helps in treating infection, rheumatism, fever and headache. Traditionally, this plant is used in various disorders such as diarrhea (Research, J & Pharmacognosy, & Lenkalapally et. al 2012), fever, inflammation, liver diseases, bronchitis, skin diseases (Upadhyay, A. et. al 2014), and malarial fever. A decoction is used to treat headaches. The seeds of *Cleome viscosa* are reported to be nutritious while the leaves juice is applied to the skin as an anti-allergen. The root of this plant is considered as a remedy for scurvy and rheumatism (A. Rajani et. al 2014). The plant parts are used in treating liver diseases, chronic painful joints (Ahmed, Salman & Sultana et. al 2011) and neurological disorders (Singh, H. et. al 2016).

The present effort is to investigate the haemolytic and antioxidant property of saponin glycosides extracted from *Cleome viscosa*.

MATERIAL AND METHODS

Chemicals and Materials:

Filter paper, reagent bottle, centrifuge, test tubes, separatory funnel, medium size beakers, wash bottle, evaporating dish, graduated cylinder, spatula, micropipette and tips, incubator, scale, stopwatch, dropper's spectrophotometer. The chemicals used in this project are SD fine chemicals.

Collection of plant material:

Fresh seeds of *Cleome viscosa* were collected from the *Cleome viscosa* plant collected from premises of Osmania University, Hyderabad, Telangana, India.

Preparation of seed powder:

The seeds of *Cleome viscosa* were dried in a shade and were grinded well using mechanical blender into fine powder and transferred into airtight glass bottles.

Extraction of Saponin glycosides from seed powder of *Cleome viscosa*:

The saponins were extracted by analytical Method from dry seed powder of *Cleome viscosa* as follows (Figure 1).

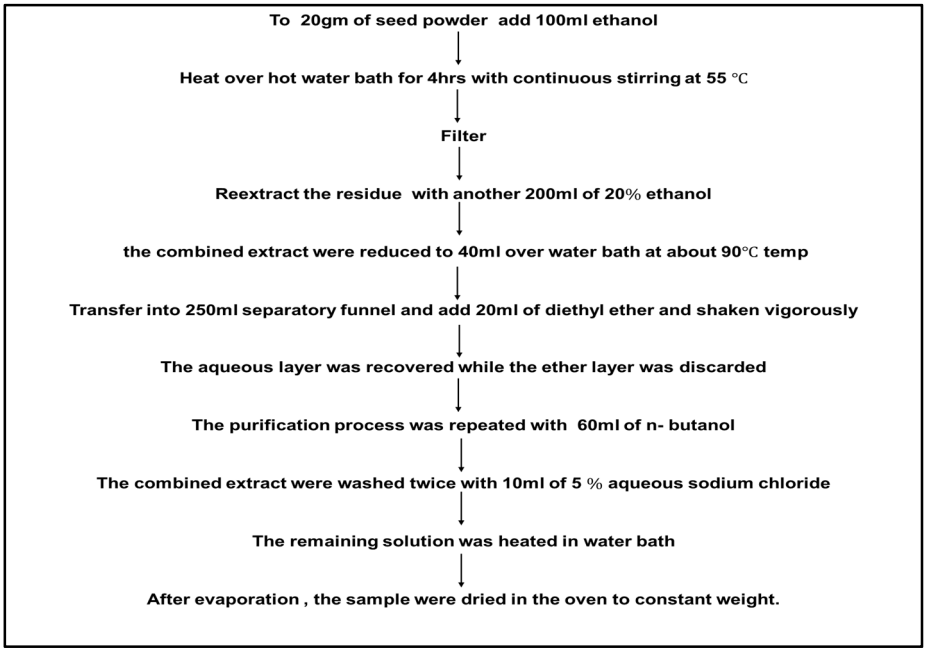

Figure 1: Extraction protocol for the extraction of saponin glycosides from seed powder of *Cleome viscosa*

Identification of Saponin glycosides extracted from the seed powder of *Cleome viscosa* by preliminary test:

i. Honeycomb test:

Dilute 5ml of the extract with water and shake vigorously.

ii. Fehling's and Benedict's test:

To extract add 1ml of diluted sulphuric acid and boil gently for 3-5min, then make the filtrate alkaline with sodium hydroxide, then perform Fehling's and Benedicts test to identify the presence of saponins.

iii. Hemolytic test:

Take a test tube in which 1ml of 10% solution of blood in normal saline was added followed by 1ml of saponin glycosides extracted from seed powder of *Cleome viscosa* and shake the tube gently and notice the result.

Identification of Saponin glycosides extracted from seed powder of *Cleome viscosa* by thin layer chromatography:

The saponins extracted from seed powder of *Cleome viscosa* have been identified by thin layer chromatography using aluminum silicate plate as a stationary phase and chloroform: methanol: distilled water (7: 4.4:1) as a mobile phase. The dilute sulphuric acid was used as a spraying reagent followed by dried the TLC plate in a hot oven at 100 ℃ for 10min to visualize the isolated saponin glycosides.

Hemolytic assay of saponin glycosides extracted from seed powder of *Cleome viscosa*:

The hemolytic activity of saponin glycosides were assessed by spectrophotometric method in which a volume of 0.5 ml of the red blood cells suspension mixed with different concentrations of saponin glycosides extracted from the seed powder of *Cleome viscosa* in phosphate buffer saline. The sample mixtures were incubated for 30min at 37°C in an incubator and then centrifuged at 1500rpm for 10min. The lysis of red blood cells and the leakage of hemoglobin was assessed by measuring the hemoglobin present in the solution freely in the supernatant at 540nm by spectrophotometer. The sodium phosphate buffer was used as a negative control and Triton X-100 was used as a positive control. The experiment was carried out and percentage hemolysis calculated according to the following formula.

$$\% \text{ Haemolysis} = (At\text{-}An/Ac\text{-}An) * 100$$

Here: At is the absorbance of the test sample (saponin glycosides). An is the absorbance of the negative control (phosphate buffer) Ac is the absorbance of the positive control (Triton X 100 (10%))

Antioxidant activity of saponin glycosides extracted from seed powder of *Cleome viscosa*:

The radical scavenging activity of the extracted saponin glycosides against the 2, 2-Diphenyl-1-picrylhydrazyl radical was determined with some modifications (Koyyati, R. et. al 2019). Different concentrations of saponin glycosides were mixed with 1mL of 2, 2-Diphenyl-1-picrylhydrazyl dissolved in methanol (9mg/100ml) and set aside in the dark (15min at room temperature). The ability to scavenge 2, 2-Diphenyl-1-picrylhydrazyl radical was measured at 517nm, as follows:

$$\textbf{Radical scavenging (\%)} = \textbf{((A0-A1)/A0) X100}$$

Where A0 and A1 are the absorbance intensities of the control and the sample, respectively.

RESULTS AND DISCUSSION

Study area and sample Collection:

The seeds were obtained from *Cleome viscosa* plant collected from premises of Osmania University, Hyderabad, Telangana, India (Figure 2).

Figure 2: Study area of *Cleome viscosa* plant and seeds

Preparation of seed powder of *Cleome viscosa*:

The seeds were dried and grinded to powder in a grinder and stored in a stable condition for further studies (Figure 3).

Figure 3: *Cleome viscosa* seed powder

Extraction of saponin glycosides from seed powder of *Cleome viscosa*:

The saponin glycosides were extracted from the seed powder of *Cleome viscosa* by analytical method (Figure 4).

Figure 4: Extraction of saponin glycosides from seed powder of *Cleome viscosa*

Identification of saponin glycosides extracted from *Cleome viscosa* by color test:

The presence of saponin glycosides in the extracted sample was identified by honeycomb test in which a froth has been observed upon shaking the extract, in Fehling's and Benedict's test a reddish precipitate was observed and in lead acetate test yellow precipitate was observed (Figure 5), Hemolytic test in which the lysis of red blood cells was identified (Figure 6).

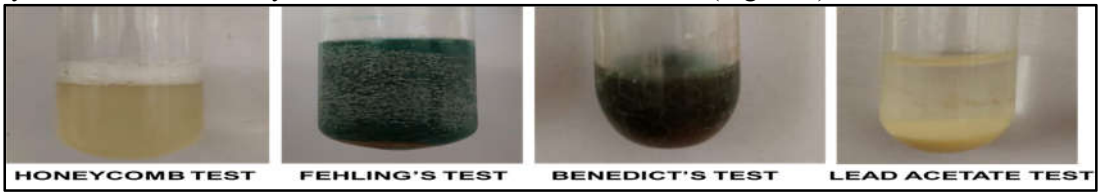

Figure 5: Identification of saponin glycosides extracted from seed powder of *Cleome viscosa*

Figure 6: Hemolytic test for the identification of saponin glycosides extracted from the seed powder of *Cleome viscosa*

Identification of saponin glycosides extracted from *Cleome viscosa* by thin layer chromatography:

After thin layer chromatographic analysis, the saponin glycosides were separated and identified efficiently (Figure 7).

Figure 7: Identification of saponin glycosides extracted from the seed powder of *Cleome viscosa* by thin layer chromatography

Hemolytic assay of saponin glycosides extracted from seed powder of *Cleome viscosa*:

The hemolytic assay was performed by spectrophotometrically measuring the amount of hemoglobin released into the solution to assess the effect of saponin glycosides extracted from seeds of *Cleome viscosa* at pH 7.4. The performance of the hemolysis assay carried out by the negative control (Phosphate buffer) and positive control (Triton-X-100). The hemolytic activity of saponin glycosides showed efficient hemolysis of red blood cells (Figure 8).

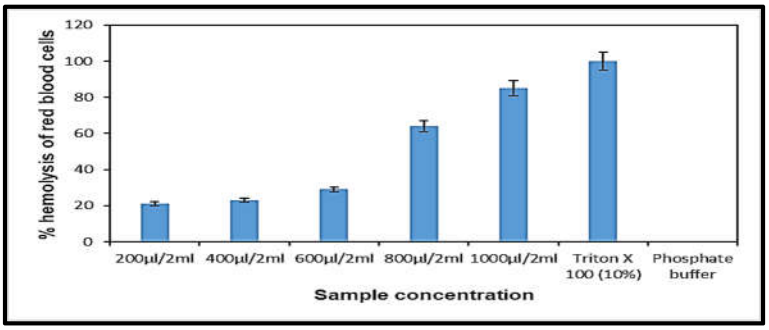

Figure 8: Graph showing the haemolytic activity of saponin glycosides extracted from the seed powder of *Cleome viscosa*

Antioxidant activity of saponin glycosides extracted from seed powder of *Cleome viscosa* by DPPH assay:

The saponin glycosides extracted from the seed powder of *Cleome viscosa* showed efficient radical scavenging activity by DPPH assay (Figure 9).

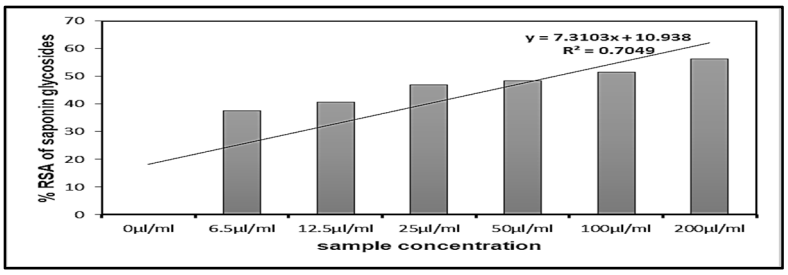

Figure 9: Graph showing the %Radical scavenging activity of saponin glycosides extracted from the seed powder of *Cleome viscosa*

CONCLUSION

. The radical scavenging activity has been widely recognized for its role in preventing oxidative damage caused by the free radicals, highly reactive species generated in the body and contribute to a myriad of diseases. However, the body's antioxidant defense system, primarily composed of antioxidants, helps neutralize these harmful radicals whereas the hemolytic potential which helps to study about the toxicity of various drugs and chemicals in blood and identify certain health problems.

In the present study, we have investigated the hemolytic and antioxidant potential of saponin glycosides extracted from the seed powder of *Cleome viscosa*. Further research is warranted to elucidate the specific mechanisms underlying their hemolytic and antioxidant activity and to explore their potential for investigating novel applications.

Conflict of interest:
The authors declare no conflict of interest.

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