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BIOLOGICAL ACTIVITY OF SAPONIN FROM MEDICINAL PLANT COLEUS FORSKOHLII BRIQ

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Abstract:
The present study looked at the saponin from the medicinal plant Coleus forskohlii Briq's antioxidant, cytotoxic, and antiheamolytic properties. Notably, numerous assays, including the DPPH (28.77µg/ml), ABTS (57.57µg/ml), LPO (64.40 µg/ml), hydrogen peroxide (76.02 µg/ml), and nitric oxide (76.02 µg/ml) tests, were used to confirm the antioxidant activity of the plant species under investigation. The breast cancer cell line MCF-7 was used to assess cytotoxic activity, and the results revealed that it was dose-dependent. With percentages of inhibition ranging from 11.15 to 61.03%, the extract's antihemolytic activity revealed a sufficient biological effect.

Keywords: Saponin; Antioxidant; Antiheamolytic; Anticancer; Coleus forskohlii

1. Introduction:

India is recognized for its rich ecosystem and boasts one of the world's most diverse plant medicinal cultures. Currently, medicinal plants are used as therapeutic agents in the developing countries, where 80% of the human population relies on plant resources for health care. Modern medicine today prefers to employ plant active components rather than complete plants (Sivaranjani et al., 2018). Secondary metabolites or phytochemicals from plants have anti-oxidative, anti-allergic, antibacterial, hypoglycemic, and anti-carcinogenic properties. These secondary metabolites protect cells from the harm produced by free radicals, which are unstable molecules (Harini et al., 2017). Saponin, as secondary metabolic product from plants are appealing candidates for novel medication research and development since their long tradition of popular medicinal usage. Chemical synthesis has proven to be an acceptable substitute for the existence of natural saponin. Saponin purchased in significant amounts by pharmaceutical businesses due to its application in the semi-synthesis of steroidal medicines for phytotherapy and in the cosmetic sector (Sharma et al., 2012). A few saponins, in particular, have an antioxidant effect (Jagadeesan J, et al., 2012) and may be particularly toxic to cancer cells (Podolak Irma, et al., 2010). ROS are ubiquitous byproducts of the cellular oxidative system and play critical roles in the regulation of cell survival, apoptosis, cell signaling, and inflammation-related factor production (Touyz et al., 2005). Several observational studies have been conducted to investigate whether the use of dietary antioxidant supplements is associated with lower cancer risks in people. In general, the findings of these investigations have been ambiguous (Patterson RE et al., 1997). Antioxidants have been experimentally demonstrated to be beneficial in a few preclinical investigations. Cholesterol-like substances are found in cancer cell membranes. Saponin, like cholesterol, can bind to these

chemicals and disrupt cancer cell proliferation. According to a study published in the Journal of Nutrition, saponin from soybeans may slow the growth of cancer cells (Rao AV, et al., 1995). Other studies have shown that saponin can induce cancer cell death and slow tumor development (Yan LL, et al., 2009). It's important to remember that most of the research into the effects of saponin on cancer cells has been preliminary, and it includes specific combinations of other extracts for effective therapeutic role. A brand-new area of investigation for pharmacological lead discovery is in vitro hemolytic activity. The erythrocyte membrane (lipid/protein) could be damaged by oxidation through various kinds of hemoglobinopathies, oxidative medicines, transition metal overload, radiation, and anomalies which can result in hemolysis (Kamila G, 2018). The hemolysis assay can be used to determine whether or not cytotoxic action is linked to actual cell membrane damage. In pursuit of promising natural chemicals, researchers have begun looking for plants that have significance from an ethno botanical perspective. These studies are important since some individuals have developed resistance to common medications like aspirin and/or conventional therapy. This affects society significantly. Additionally, the widespread use of synthetic drugs poses a serious threat to society. There is a growing need for pharmaceuticals derived from plant sources, and there is established interest in traditional medicine these days. This resurgence in interest in plant-based medications is largely attributable to the widespread perception that "Green medicine" is safer and more dependable than pricey manufactured medications, many of which have negative side effects. According to Bone K. (2007), the Lamiaceae family includes the common indigenous medicinal plant Coleus forskohlii Briq (Syn. Plectranthus barbatus Andr.). The tuberous C. forskohlii, C. amboinicus, C. blumei, and C. malabaricus are the four main medicinal Coleus plant species found in India; the majority of these plants are used to treat digestive problems and diarrhoea (De Souza NJ et al., 1993). The root extract of Coleus forskohlii has powerful medicinal effects against a wide range of conditions, including cephalalgia, diabetes, arthralgia, arthritis, hemorrhoids. The dried stem is used for blood, ulcers, inflammations, erysipelas, skin illnesses, and rheumatism, while the roots are used for laxative, analgesic, rheumatism, dropsy, paralysis, and intestinal ulcers (Kavitha C et al., 2010). However, no systematic work has been undertaken on analyzing the saponin from Coleus forskohlii for antioxidant, antimicrobial and anticancer activities. Hence the present study was formulated to evaluate the in vitro free radical scavenging activity (antioxidant property), in vitro antiheamolytic, anticancer and cytotoxic property of saponin from Coleus forskohlii.

2 Materials and Methods:

2.1 Collection and Identification of plants: Plectranthus barbatus was gathered and identified using molecular characterization in the investigation. Plectranthus barbatus was gathered at random from the Kolli Hills region. Plectranthus barbatus was air-dried before being ground into a fine powder and stored in an airtight bottle. The extracts were then vacuum-dried and stored in a refrigerator.

2.2 Isolation, separation, and purification of saponin from Plectranthus barbatus roots: In a Soxhlet extractor, powdered plant material was extracted using consecutive solvent treatments. Petroleum ether or n-hexane was used to remove fat from the powdered material. Methanol was then used to remove the defatted substance. The methanolic extract is concentrated (ideally under vacuum by rotational evaporation) to produce dry extract. Dried methanol extract was suspended in distilled water and agitated with n-butanol, and then the crude saponins mixture was precipitated by adding solvent ether. The saponin estimation and purification were done and reported in Kabilraj et al., 2022.

2.3 Antioxidant and free radical scavenging analysis of saponin from Coleus forskohlii: The saponin of Coleus forskohlii was tested in vitro for its effectiveness.

2.3.1 DPPH radical scavenging activity: The DPPH assay was done by following the Blois et al., (1958) method. To 2 ml of a methanolic solution of 0.2 mM DPPH, 0.2 ml of saponin extract was added at varying concentrations 250, 200, 150, 100, 50g/ml. The reaction mixture was mixed well and incubated for 30 minutes at 37ºC under dark condition. The control contains 0.2 ml methanol and 2.8 ml DPPH. The DPPH radical scavenging activity was determined by measuring absorbance at 490 nm using microplate reader. The percentage of radical scavenging activity of the sample was calculated using the formula as given below:

Radical Scavenging Activity % =1 – (absorbance of sample)/ (absorbance of control) X 100

2.3.2 Reducing power assay: The concept of this assay is that the higher the level of the absorbance, the greater the reducing power. 2 mg of each extract and the standard - ascorbic acid - were accurately weighed and diluted in 2 ml of DMSO. After that, 0.5 ml of the aforesaid solution was diluted with phosphate buffer (0.2 M, pH 6.6). Lower dilutions were made in a series with DMSO. Oyaizu's 1986 approach for reducing power test was used. The above sample was treated with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was then placed in a 50°C water bath for 20 minutes. The resultant solution was immediately chilled, mixed with 2.5 mL of 10% trichloroacetic acid, and centrifuged at 3000 rpm for 10 minutes. After that, 5 mL of supernatant was combined with 5 mL of distilled water and 1 mL of 0.1% ferric chloride. After 10 minutes of treatment, absorbance at 700 nm was measured.

2.3.3 ABTS radical scavenging activity: The extract and the standards, ascorbic acid and rutin, were carefully weighed and diluted individually in 2ml of DMSO. To obtain the lower dilutions, these solutions were serially diluted using DMSO. ABTS (54.8 mg) was diluted to a 2 mM concentration in 50 ml of distilled water, and then potassium persulphate (17 mM, 0.3 ml) was added. Before use, the reaction mixture was allowed to stand at room temperature in the dark overnight. In order to obtain a final amount of 1.36 ml, 1.0 ml of distilled DMSO and 0.16 ml of ABTS solution were added to 0.2 ml of varied strengths of the extract or standards. After 20 minutes, the absorbance of the sample was obtained spectrophotometrically at 734 nm.

2.3.4 Hydrogen peroxide scavenging assay: Ruch et al.'s approach was used to determine hydrogen peroxide scavenging activity. The quantities of hydrogen peroxide (40 mM) were quantified spectrophotometrically at 230 nm in phosphate buffer (pH 7.4). Extracts (25-50 g/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40 mM), and the absorbance of hydrogen peroxide at 230 nm was measured after 19 minutes against a blank solution in phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging activity by extracts and standard compounds was estimated using the following equation:

[H2O2] % scavenged = [(A0-A1)/A0] x 100 A0 represented the absorbance of the control, while A1 represented the absorbance of root extracts or standards.

2.3.5 Nitric oxide radical scavenging activity: Sodium nitroprusside was used in the nitric oxide scavenging experiment. This can be determined by employing the Griess Illosvoy reaction. 2 ml of 10 mM sodium nitroprusside in 0.5 ml of phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of extract/sub-fraction at various concentrations and incubated at 25°C for 150 minutes. 0.5 mL of the incubated mixture was taken out and put to 1.0 mL of sulphanilamide solution (0.33% in 20% glacial acetic acid) and incubated at room temperature for 5 minutes. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and kept at room temperature for 30 minutes. At 546 nm, the absorbance was measured. A typical blank/control solution had the

same solution mixture as the test solution but with no plant extract or standard. At 546 nm, the absorbance of the blank/control solution was measured. The % inhibition was estimated using the following formula: Where Al denotes the absorbance of the extract or standard, and A0 denotes the absorbance of the control.

2.4 Antihemolytic assay: Blood was drawn from healthy adult human volunteers and stored in sterile Alsevier's solutions before being used within 5 hours. A volume of 800 µl of 1% w/v Triton X-100 was dilute it with phosphate buffer to a level of 3 ml in a series of test tubes. Similarly, 3 mL of pure water served as the sole positive control. Different concentrations of plant extracts (100-500 g) were added to a series of tubes that had previously been incubated with Triton X-100. 500 l of RBC suspension was added to each tube and gently stirred. Tubes were incubated in a 37ºC water bath for 1 hour before being centrifuged at full speed for 5 minutes. The supernatant was collected, and the absorbance at 541 nm was measured against a blank of phosphate buffer to calculate the percentage of hemolysis (Oguiura N et al., 2011).

2.5 Anticancer activity of saponin from Coleus forskohlii - MTT assay: Using MCF-7 cells, the sample was examined for in vitro cytotoxicity using the 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, trypsinized MCF-7 cells were collected and pooled in a 15 ml tube. The cells were then seeded into 96-well plate in DMEM media with 10% FBS and 1% antibiotic solution for 24-48 hours at 37°C. The wells were rinsed with sterile PBS before being treated with a variety of sample concentrations in a serum-free DMEM medium. Each sample was duplicated three times, and the cells were cultured for 24 hours at 37°C in a humidified 5% CO2 incubator. Following the incubation period, MTT (20 L at 5 mg/ml) was added to each well, and the cells were incubated for another 2-4 hours until purple precipitates were clearly visible under an inverted microscope. Finally, the medium and MTT (220 L) were aspirated from the wells and washed with 1X PBS (200 L). In order to dissolve the formazan crystals, 100 µL of DMSO was added and the plate was agitated for 5 minutes. The absorbance of each well was measured at 570 nm with a micro plate reader (Thermo Fisher Scientific, USA), and the % cell viability, IC50, and CTC50 values were calculated with Graph Pad Prism 6.0 software (USA).

RESULTS AND DISCUSSION

The antioxidant potential has been evaluated in the present study by five different radical scavenging studies, including DPPH, ABTS, LPO, hydrogen peroxide, and nitric oxide. The antioxidant activity of saponin from Coleus forskohlii was examined at various concentrations ranging from 20 to 100 g/ml. The free radicals were scavenged by the test compounds in a concentration-dependent way up to the specified concentration in all the models, according to the observed percentage of inhibition**.**

DPPH radical scavenging assay:

The activity of saponin from Coleus forskohlii and rutin in scavenging DPPH radicals was determined. The IC50 values for DPPH scavenging activity of saponin from Coleus forskohlii and standard rutin were found to be 28.77µg/ml and 21.40µg/ml, respectively, as shown in Fig. 1. The higher inhibition activity was recorded in Coleus Forskohlii in a dose-dependent manner compared to standard rutin. Herbal remedies might be effective in treating a variety of illnesses because of their antioxidant properties. By scavenging hydroxyl radicals, the saponin from Coleus Forskohlii in the current study was able to prevent 2-deoxy-D-ribose from being oxidatively degraded. Rutin's antioxidant action has an excellent record of effectiveness (Ajay Mandal et al., 2015).

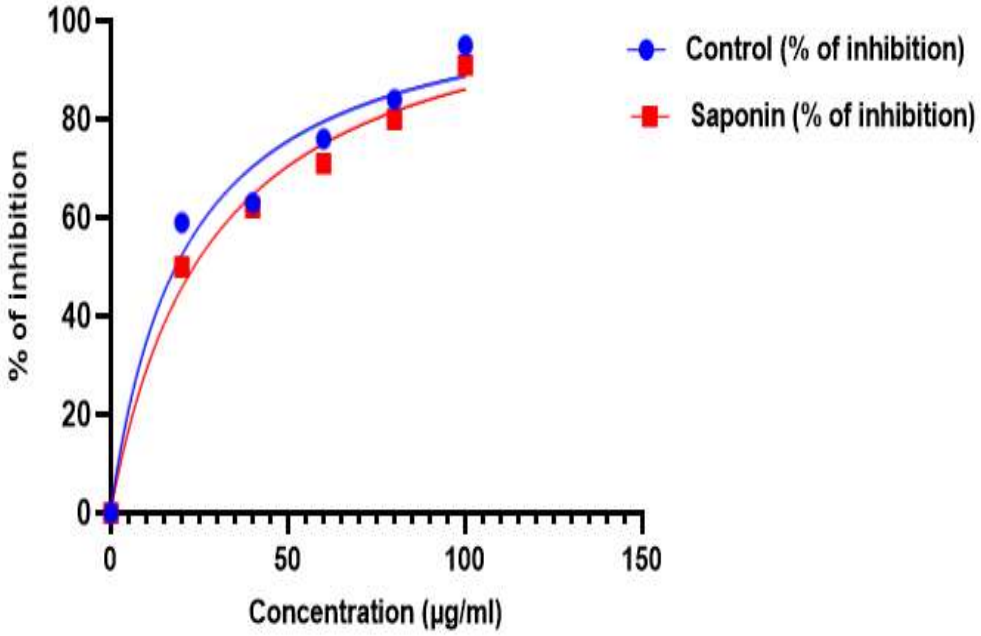

Figure 1: DPPH radical scavenging activity of saponin from Coleus forskohlii

LPO Scavenging Activity Assay:

Saponin from Coleus forskohlii and rutin had IC50 values for LPO scavenging activity of 64.40 µg/ml and 53.53µg/ml, respectively as shown in figure 2. Coleus forskohlii's greater inhibitory effect was observed in a dose-dependent manner. Free radical production is a physiological process that occurs regularly and has a variety of impacts. But as these free radicals are produced more often, the lipids become more vulnerable to lipid peroxidation. Malondialdehyde (MDA), which is determined via a thiobarbiturate assay, is a frequently used, reliable indicator of lipid peroxidation. Additionally, the cells have a number of antioxidant defenses that work in opposition, according to evolution. According to Rosey Lekhru et al. (2014), these antioxidant defense mechanisms can be divided into free radical scavengers and chain-breaking antioxidants.

Nitric Oxide Scavenging Activity:

Coleus forskohlii and rutin saponin had IC50 values for nitric oxide scavenging activity of 76.02 µg/ml and 61.15µg/ml, respectively as shown in figure 3. In a dose-dependent manner, greater inhibitory activity was seen in Coleus forskohlii. The saponin markedly reduced the generation of nitric oxide from sodium nitroprusside in the mixture. With a rise in extract concentration, inhibition grew. Nitric oxide, a byproduct of free radicals in mammalian cells, has a role in the control of several physiological processes. However, according to Ponvinobala et al. (2012), excessive NO generation is linked to a number of disorders. In the current investigation, saponin from Coleus forskohlii was employed to diminish the nitrite that was formed when solutions of sodium nitroprusside were incubated at 25°C in a standard phosphate buffer. This might be as a result of the extract's antioxidant principles, which compete with oxygen to react with nitric oxide and so prevent the formation of

nitrite.

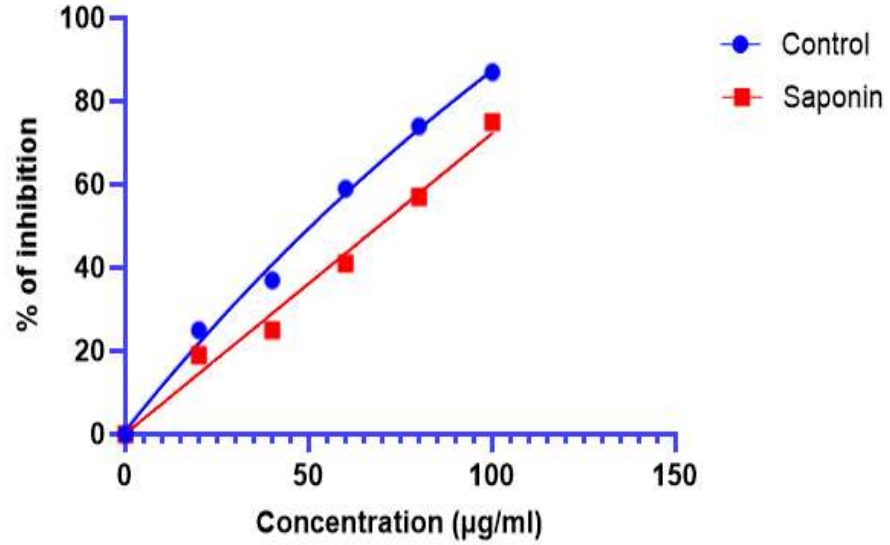

Figure 2: LPO radical scavenging activity of Saponin from Coleus forskohlii

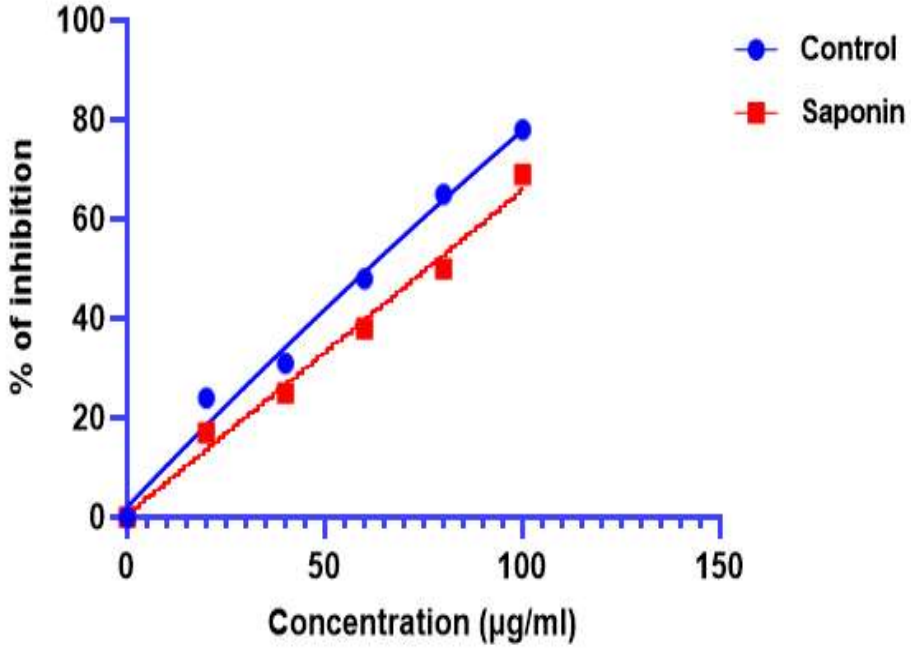

Figure 3: Nitric Oxide radical scavenging activity of saponin from Coleus forskohlii

H_2O_2 Scavenging Activity:

Saponin from Coleus Forskohlii and rutin had IC50 values for hydrogen peroxide scavenging activity of 78.03 g/ml and 63.58µg/ml, respectively as shown in figure 4. Furthermore, it was found that UA could exhibit significant antioxidant properties in the TRAP/TAR and OH radical-scavenging investigations by Rabelo et al.,

(2014) that tested the UA redox properties towards various reactive species (RS) generated in vitro and assessed its action on SH-SY5Y neuronal-like cells upon exposure to hydrogen peroxide (H2O2).

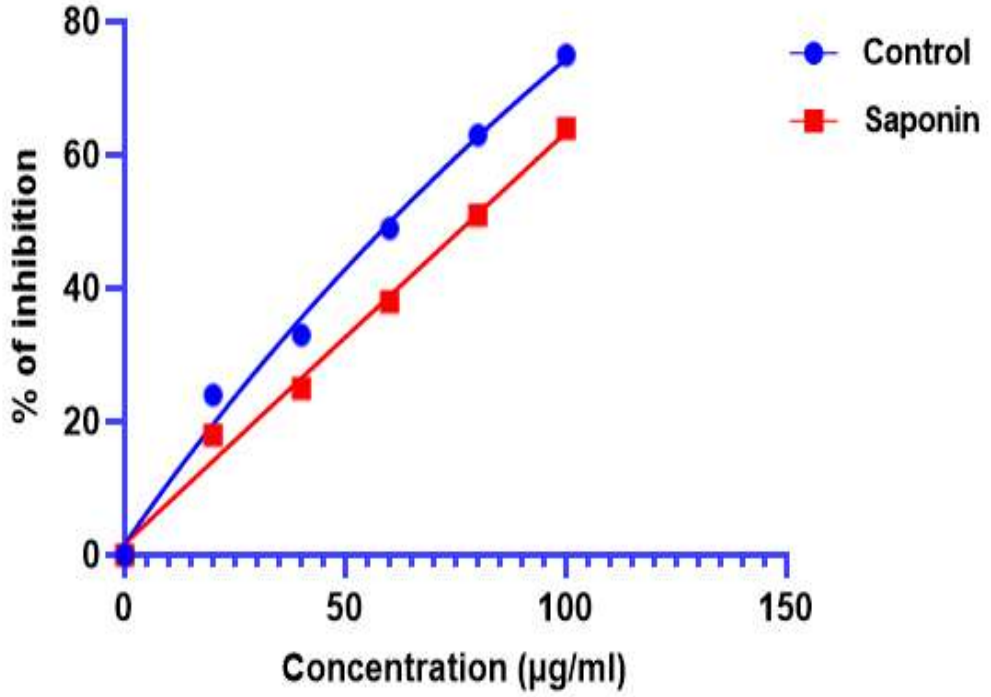

Figure 4: H_2O_2 radical scavenging activity of saponin from Coleus forskohlii

ABTS Scavenging Activity:

The antioxidant activity of individual chemicals and other complex mixtures has been assessed using the ABTS radical cation, a common organic radical (Zhou et al., 2004). One clear technique to assess the antioxidant capacity of phenolic compounds is through the decolorization of the ABTS•+ cation radical. Recent research by Awika et al. (2003) demonstrated positive relationships between phenolic content and antioxidant activity measured by the 1,1-diphenyl-2-picrylhydrazyl (DPPH), ABTS, and ORAC tests. Therefore, a compound's power to scavenge the ABTS•+ radical can be used to determine its ability to absorb oxygen radicals. The IC50 values for ABTS scavenging activity for saponin from Coleus Forskohlii and rutin were 57.50µg/ml and 42.94 µg/ml respectively as shown in figure 5. Coleus forskohlii's greater inhibitory activity has been observed in a dose-dependent manner. The current study's findings demonstrated that the saponin from Coleus forskohlii exhibited extremely powerful ABTS radical scavenging action. The plant extract was found to have a notable amount of ABTS+ radical scavenging activity; this suggests that the plant extract, particularly at higher concentrations, may be beneficial for treating pathological damage connected to radicals (Wang et al., 1998).

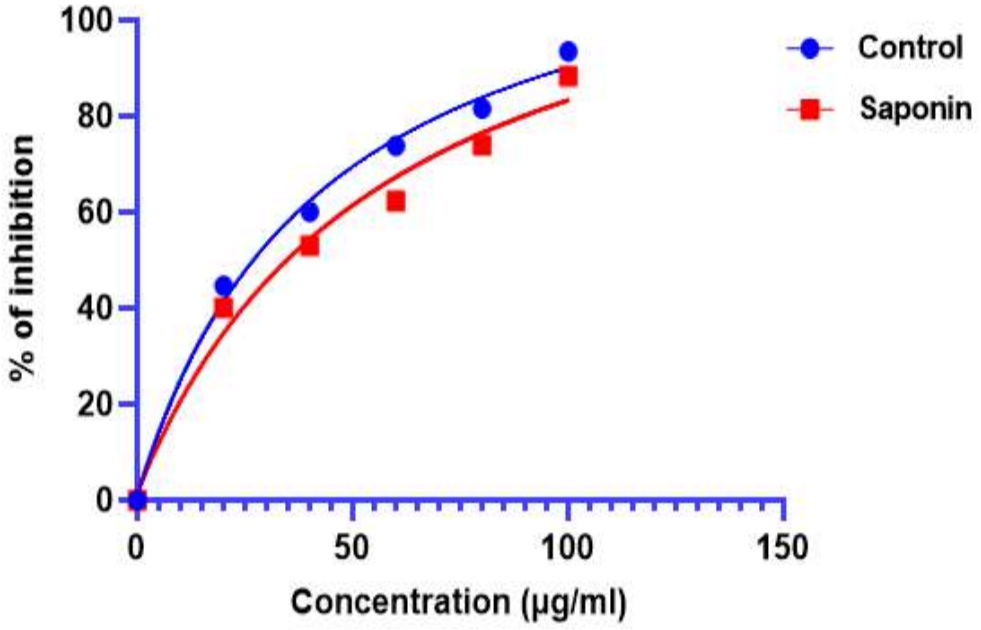

Figure 5: ABTS radical scavenging activity of saponin from Coleus forskohlii

Antihemolytic activity of saponin from Coleus forskohlii:

Red blood cells (RBCs) are the primary targets of free radicals, owing to their high membrane concentrations of polyunsaturated fatty acids (linoleic and arachidonic acids in particular) and O_2 transport associated with redox-active hemoglobin molecules, which are potent promoters of ROS. Oxidation depletes membrane protein content, deforms RBCs, and disturbs microcirculation. It is also implicated in hemolysis (Khalili Masoumeh et al., 2014). The destruction of erythrocytes causes hemolysis, which destroys haemoglobin and other internal cells. Components are released into the surrounding fluids. Due to the preponderance of polyunsaturated fatty acids in erythrocyte membranes, they are highly susceptible to oxidative damage, whose consequence is the hemolytic process (Divya et al., 2012.). In addition, oxidative stress and inflammation are evidently noticed even in malaria-associated hemolysis (Suthin et al., 2014).

Saponin from Coleus forskohlii was also capable of scavenging hydrogen peroxide in a concentration-dependent manner as recorded in table 1. Investigations were made into the saponin from Coleus forskohlii's ability to prevent hemolysis caused by H_2O_2. Overall, the extract demonstrated sufficient antihemolytic action, with percentages of inhibition ranging from 11.15 to 61.03%. An excellent model for oxidative damage to biological membranes is the oxidation of erythrocytes. Hemolysis was significantly reduced under the effect of extracts when applied to red blood cells. This might be a result of the bioactive chemicals in the extracts' capacity to scavenge free radicals (Paulsamy et al. 2013).Unsaturated fatty acids like linoleic acid and arachidonic acid undergo lipid peroxidation. Hence, membrane lipids, which contain unsaturated fatty acids, are involved in the oxidation process. Saponin from Coleus forskohlii potentially controls the oxidation of unsaturated fatty acids. Higher concentrations of polyunsaturated fatty acids (PUFA) in membranes and oxygen transport associated with

redox-active hemoglobin molecules lead to a free radical attack on erythrocytes. Singlet oxygen and hydroxyl radicals formed from superoxide anion initiate lipid peroxidation. Thus, superoxide is indirectly involved in lipid peroxidation. Reactive oxygen species promote free radical attack, resulting in lipid peroxidation. The saponin from Coleus forskohlii showed good activity in hemoglobin-induced linoleic acid systems due to the presence of high total phenolic content in the extract and thus acting as potential antihemolytic extract.

Table 1: Antihemolytic activity of saponin from of Coleus forskohlii

S. no.	Concentration of Plant Extract (µg)	Antiheamolytic Activity (%)
1	100	66
2	80	49
3	60	34
4	40	21
5	20	14

Anticancer properties of Saponin from Coleus forskohlii:

MTT tests were utilized in the current work to evaluate the cytotoxicity of the saponin from the root of Coleus forskohlii. The outcomes from the cytotoxicity investigations performed on human breast adenocarcinoma (MCF-7) revealed some degree of cell-type discrimination. The strong cytotoxic effect towards MCF-7 has been shown by the toxic effects of the saponin from Coleus forskohlii root extract on the cancer cell (figure 6). Additionally, it was shown that the cytotoxic activity was dose-dependent; the higher the concentration, the more activity was observed (figure 7). Previous research has demonstrated that the presence of diverse phytochemicals, which are separated by using proper extraction procedures, determines the biological activity of plant extracts (Paun et al., 2012; Lim, 2015).

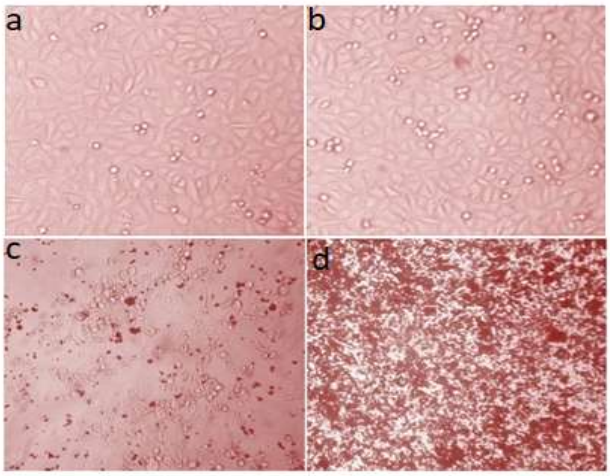

Figure 6: Cytotoxicity activity of saponin against MCF-7: Microscopic images of cells treated with saponin at varying concentrations a) Control; b) 50 µg; c) 250 µg; d) 500 µg;

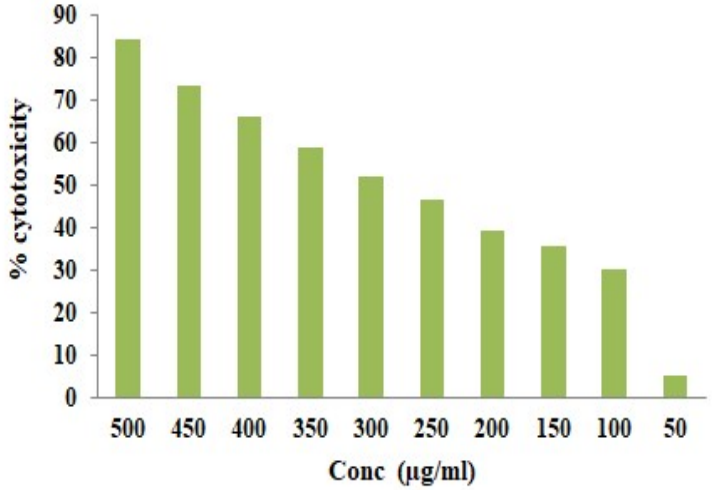

Figure 7: Graph shows the percentage of MCF-7 cell viability at different concentrations of saponin from Coleus forskohlii

Conclusion:

Using a battery of in vitro assays, various extracts of Coleus Forskohlii were studied for their antioxidant status, free radical quenching, anticancer properties, and mechanism of action. Many common plant-based meals and herbs include potent phytochemical compounds that can benefit human health. Coleus forskohlii may be deduced to have high antioxidant and free radical scavenging properties. These in vitro tests show that Coleus forskohlii is a large source of natural antioxidants, which may be useful in avoiding the advancement of various oxidative stressors.

Coleus forskohlii saponin were studied and found to have antihemolytic action. The cytotoxicity experiment revealed that Coleus forskohlii has the potential to be a source of anticancer therapeutic agents against MCF-7 cell lines. Saponins from the root of Coleus forskohlii have been shown to have strong anti-cancer effect against cancer cell lines. This could be owing to the presence of active phytoconstituents, which has been reported. As a result, this plant extract may have clinical and therapeutic potential for the most deadly diseases, such as cancer. With the rate at which cancer is spreading, it appears that urgent and successful initiatives to promote human health are required. As a result, there is a need to capitalize on the potential of these plants, particularly in traditional medicine and the pharmaceutical industries.

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References:

1. Audomkasok S, Singpha W, Chachiyo S, Somsak V. Antihemolytic activities of green tea, safflower, and mulberry extracts during Plasmodium berghei infection in mice. Journal of Pathogens. 2014.
2. Awika, Joseph M., et al. "Screening methods to measure antioxidant activity of sorghum (Sorghum bicolor) and sorghum products." Journal of agricultural and food chemistry 51.23 (2003): 6657-6662.
3. Blois, Marsden S. "Antioxidant determinations by the use of a stable free radical." Nature 181 (1958): 1199-1200.
4. Bone, Kerry. "Coleus: a new development in the fight against Metabolic Syndrome X." Townsend Letter: The Examiner of Alternative Medicine 286 (2007): 56-63.
5. De Souza, Noel J. "Industrial development of traditional drugs: the forskolin example a mini-review." Journal of ethnopharmacology 38.2-3 (1993): 167-175.
6. Divya, Nagamani, J. E. Nagamani, and S. Prabhu. "Antioxidant and antihemolytic activities of Bombax Ceiba pentandra spike and fruit extracts." International Journal of Pharmacy and Pharmaceutical Sciences 4.Suppl 5 (2012).
7. G. Paun, E. Neagu, C. Litescu, P. Rotinberg, L. Radu. Membrane processes application on the Symphytum officinale and Geranium robertianum extracts concentration to obtain high antioxidative activity compounds. Journal of the Serbian Chemical Society, 77 (2012), pp. 1191-1203.
8. Jagadeesan J, Nandakumar N, Rengarajan T, Balasubramanian MP. Diosgenin, a steroidal saponin, exhibits anticancer activity by attenuating lipid peroxidation via enhancing antioxidant defense system during NMU-induced breast carcinoma. Journal of Environmental Pathology, Toxicology and Oncology. 2012; 31(2).
9. Kabilraj, Uthra Selvaraj, and Asha Monica. Molecular Characterization of antibiogram activities and Bioactive Compound Saponin from the root of Plectranthus barbatus. Bull. Env.Pharmacol. Life Sci., Vol 11[10] September 2022 : 130-137
10. Kamila, Gheffour. "In Vitro Antihemolytic Activity of Echinops spinosus Tannins Extracts against Human Erythrocytes." Asian Journal of Pharmaceutics (AJP) 12.03 (2018).
11. Kavitha, C., K. Rajamani, and E. Vadivel. "Coleus forskohlii: a comprehensive review on morphology, phytochemistry and pharmacological aspects." J Med Plants Res 4.4 (2010): 278-85.
12. Khalili, Masoumeh, Mohammad Ali Ebrahimzadeh, and Yaghoub Safdari. "Antihaemolytic activity of thirty herbal extracts in mouse red blood cells." Archives of Industrial Hygiene and Toxicology 65.4 (2014).
13. Mandal, Ajay, and P. Jagan Mohan Reddy. "Evaluation of in vitro antioxidant activity and total phenolic content of ethanol and aqueous extracts of polyherbal formulation." (2015).
14. Oguiura, Nancy, et al. "In vitro antibacterial and hemolytic activities of crotamine, a small basic myotoxin from rattlesnake Crotalus durissus." The Journal of antibiotics 64.4 (2011): 327-331.
15. Patterson RE, Kristal AR, Glanz K, McLerran DF, Hebert JR, Heimendinger J, Linnan L, Probart C, Chamberlain RM. Components of the working well trial intervention associated with adoption of healthful diets. American Journal of Preventive Medicine. (1997);13(4):271-6.
16. Paulsamy S, Moorthy D, Nandakumar K, Saradha M. Evaluation of in vitro antioxidant potential of methanolic extracts of the ferns, Actiniopteris radiata (Sw) Link. and Equisetum ramosissimum Desf. Int J Res Dev Pharm L Sci. (2013); 2(3):451- 455.
17. Podolak, Irma, Agnieszka Galanty, and Danuta Sobolewska. "Saponins as cytotoxic agents: a review." Phytochemistry Reviews 9 (2010): 425-474.

18. Ponvinobala K., Kanchana G., and Rubalakshmi G. In vitro antioxidant activity of hydroalcoholic extract of Andrographisneesiana leaves.Journal of Pharmacy Research, (2012); 5(2): 1256-1259.
19. Rabelo S.C.; Amezquita Fonseca, N.A.; Andrade R.A.; Maciel R.; Costa A.C. Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide, Biomass and bioenergy, (2011); 35, 2600-2607.
20. Rao, A. V., and M-K. Sung. "Saponins as anticarcinogens." The Journal of nutrition 125.suppl_3 (1995): 717S-724S.
21. Ruch, R.J., Cheng, S.J., and Klaunig, J.E. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis, (1989); 10, pp. 1003-1008.
22. Sharma P, Jha AB, Dubey RS, Pessarakli M. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. Journal of botany. 2012.
23. Sivaranjani Ganapathy, Dr. R. Suja, Dr. P. G. Latha, Dr. B. S. Bijukumar, Dr. V J Shine, Dr. P Shikha, Dr. Sreejith G. **"Phytochemical analysis, Anti-inflammatory and Analgesic activity of Vitex altissima L.f. stem bark."** International Journal of Advance Research, Ideas and Innovations in Technology 4.4 (2018). www.IJARIIT.com.
24. T.K. Lim, Edible Medicinal and Non-Medicinal Plants. Springer Netherlands, Dordrecht (2015)
25. Touyz, Rhian M. "Reactive oxygen species as mediators of calcium signaling by angiotensin II: implications in vascular physiology and pathophysiology." Antioxidants & redox signaling 7.9-10 (2005): 1302-1314.
26. Wang, M., Li, J., Rangarajan, M., Shao, Y., La Voie, EJ. Huang, T., Ho, C. Ant oxidative phenolic compounds from sage (Salvia officinalis)**.** J AgricFood Chem, (1998); **46**:4869-4873.
27. Yan LL, Zhang YJ, Gao WY, Man SL, Wang Y. In vitro and in vivo anticancer activity of steroid saponins of Paris polyphylla var. yunnanensis. Experimental Oncology. 2009.
28. Zhou, Kequan, and Liangli Yu. "Effects of extraction solvent on wheat bran antioxidant activity estimation." LWT-Food science and Technology 37.7 (2004): 717-721.