International Journal of Herbal Medicine
Available online at www.florajournal.com

E-ISSN: 2321-2187
P-ISSN: 2394-0514
www.florajournal.com
IJHM 2024; 12(2): 10-15
Received: 24-12-2023
Accepted: 28-01-2024

Komla Mawunyo Dossouvi
A) Viroscope SARL-U, Lomé, Togo
B) Department of Microbiology, DGlobal Health Research Institute, Lomé, Togo

Kossi Jean Dossouvi
Viroscope SARL-U, Lomé, Togo

Akueba Landrine Dossouvi
Viroscope SARL-U, Lomé, Togo

Kokou Dossouvi
Viroscope SARL-U, Lomé, Togo

Corresponding Author:
Komla Mawunyo Dossouvi
A) Viroscope SARL-U, Lomé, Togo
B) Department of Microbiology, DGlobal Health Research Institute, Lomé, Togo

VIROSCOPE®, an ethanolic extract of medicinal plants produced in Lomé, Togo, demonstrated excellent anti-inflammatory and immune cell proliferation activities

Komla Mawunyo Dossouvi, Kossi Jean Dossouvi, Akueba Landrine Dossouvi and Kokou Dossouvi

DOI: https://doi.org/10.22271/flora.2024.v12.i2a.925

Abstract
The World Health Organization (WHO) estimated that at least 80% of the world's population finds its main, if not exclusive, therapeutic source in plants. Inflammation and associated diseases have been reported to be a great threat to public health, as evidenced by the deadly cytokine storms observed during the latest COVID-19 pandemic. Several phytomedicines with excellent anti-inflammatory and immunostimulatory activities have been reported worldwide. Thus, this study evaluated the *in vitro* cytotoxicity, immune cell proliferation and immunosuppressive activities of a phytomedicine "VIROSCOPE®" produced by Viroscope SARL-U in Lomé, Togo. Cytotoxicity was assessed using the Propidium Iodide Membrane Integrity Test and interpreted according to NF EN ISO 10993-5. The immune cell proliferation property was assessed by evaluating the expression of the proliferating cell nuclear antigen (PCNA) in white blood cells by flow cytometry. Evaluation of IL-6 and TNF α production by Sandwich ELISA was used to evaluate the immunosuppressive and immunostimulatory properties of VIROSCOPE®. VIROSCOPE® demonstrated slight and mild cytotoxicity, depending on the dose. In addition, VIROSCOPE® induced excellent *in vitro* immune cell proliferation and showed excellent anti-inflammatory activity. All these immunomodulatory activities were dose-dependent, calling for strict compliance with the dosage. Further *in vitro* studies, animal model tests, and clinical trials are needed to elucidate all the therapeutic activities of VIROSCOPE® and to improve its formulation.

Keywords: VIROSCOPE®, Phytotherapy, anti-inflammatory, cytotoxicity, immune cell proliferation

1. Introduction
Phytotherapy is the science of treating and preventing diseases using medicinal plants and herbal products. Recently, the World Health Organization (WHO) estimated that at least 80% of the world's population finds plants as their main, if not exclusive, therapeutic source [1].
In fact, since prehistoric times, in ancient Mesopotamia, ancient China, ancient India, ancient Egypt, ancient Greece, ancient Rome, Arabia and Persia, plants had always been used successfully to treat all kinds of diseases [2, 3].
Phytomedicines contain phytocomplexes, which are complexes of active molecules with specific biological activities. The biological activity of a phytocomplex is generally stronger than the sum of the activities of the individual active molecules, and the presence of substances with no specific activity can have a significant synergistic effect [4, 5].
Inflammation and associated diseases have been reported to be a great threat to public health, as evidenced by the deadly cytokine storms often observed in influenza and COVID-19 [6–9].
Pro-inflammatory cytokines are mainly produced by activated macrophages and participate in the positive regulation of inflammatory reactions. Interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) are major pro-inflammatory cytokines involved in the pathological pain process [10]. Additionally, many degenerative disorders and chronic health conditions including arthritis, diabetes, obesity, cancer, and cardiovascular diseases are associated with the emergence of a low-grade inflammatory state. Phytomedicines are good immunosuppressive and immunomodulatory candidates. Indeed, phytochemicals often act via regulatory molecular mechanisms that synergize anti-inflammatory pathways, such as increased production of anti-inflammatory cytokines or interference with inflammatory pathways by reducing the production of pro-inflammatory cytokine modulators [11–14].

Leukocyte proliferation is the first step towards achieving an adequate immune response. However, many health conditions and viral infections, such as HIV and HCV infections, cause severe leukopenia and patients with severe leukopenia become fertile grounds for all types of secondary infections. Several conventional medications for severe leukopenia are effective but are also very expensive and cause many side effects. Therefore, identifying alternative medications is necessary. Several phytomedicines have demonstrated immune cell proliferation potential in the treatment of severe leukopenia [15–17].

Despite these past exploits and the promising future that herbal medicine seems to offer, some issues must be addressed. First, the preparation processes for each phytomedicine and traditional plant-based product must be standardized. Additionally, every traditional herbal medicine must undergo *in vitro* quality control testing, animal model testing, and clinical trials to prove their effectiveness, adjust their dosages, and address possible toxicity issues [18–20].

Thus, this study aimed to evaluate the *in vitro* cytotoxicity, immune cell proliferation and immunosuppressive properties of the phytomedicine "VIROSCOPE®" produced by Viroscope SARL-U in Lomé, Togo.

1.1 Presentation of VIROSCOPE®

VIROSCOPE® is an ethanolic extract of medicinal plants produced by Viroscope SARL-U, Lomé, Togo, and is a dark yellow liquid with a medium consistency. VIROSCOPE® is prepared using *Sodabi* (a traditionally distilled palm wine with a total alcohol content of 42%) as solvent. Ethanol is the main alcohol found in *Sodabi*, with a proportion of 99%v/v. After preparation, VIROSCOPE® has a final alcohol content of 30%. VIROSCOPE® is administered orally. The dosage of VIROSCOPE® for an adult human over 18 years of age is 40 ml twice a day (morning and evening), whereas the dosage of VIROSCOPE® for a human aged between 7 and 18 years is 20 ml twice a day (morning and evening), and the dosage for a child aged between 3 and 8 years is 05 ml twice a day (morning and evening). VIROSCOPE® is prohibited for children under three years of age and is authorized for pregnant and breastfeeding women. Previous studies had detected the presence of phytochemical groups present in VIROSCOPE® (polyphenols, hydrolyzable tannins, flavonoids, alkaloids and saponosides) and characterized rutin (195.37 ± 6.01 mg/g of dry extract) and gallic acid (121.31 ± 2.49 mg/g of dry extract). Additionally, other previous studies of VIROSCOPE® have characterized minerals, including calcium (8216 ± 1 mg/kg), magnesium (10389 ± 1 mg/kg), iron (83 ± 1 mg/kg), and zinc (19 ±1 mg/kg).

2. Materials and methods

2.1 Assessment of cytotoxicity

The method used involved direct contact with the cell culture on a microplate at 37°C under CO2 for 24 h. Membrane integrity was assessed by flow cytometry using propidium iodide (PI). Human peripheral blood white cells were used, and the cell mortality rate was determined for the positive control, negative control, and VIROSCOPE®. The doses of VIROSCOPE® 40 ml, 20 ml and 5 ml were tested, and each sample was tested twice. The interpretation of the cytotoxicity evaluation was according to NF EN ISO 10993-5 "Biological evaluation of medical devices - Part 5: test concerning *in vitro* cytotoxicity".

2.2 Evaluation of immune cell proliferation

The method used was direct contact with cell culture on a microplate at 37°C under CO2 for 24 h. Human peripheral white blood cells were used, and the expression of the proliferating cell nuclear antigen (PCNA) in negative control, positive control and cells sensitized with VIROSCOPE® were evaluated by flow cytometry. The doses of VIROSCOPE® 40 ml, 20 ml and 5 ml were tested, and each sample was tested twice.

2.3 Evaluation of immune stimulating properties

The method used was direct contact with cell culture on a microplate at 37°C under CO2 for 24 h. The technique used was the evaluation of the production of IL-6 and TNF α by Sandwich ELISA for the positive control, negative control and VIROSCOPE®. The doses of VIROSCOPE® 40 ml, 20 ml and 5 ml were tested, and each sample was tested four times.

2.4 Evaluation of immunosuppressive properties

The method used was direct contact with cell culture on a microplate at 37°C under CO2 for 24 h. The technique used was the evaluation of the production of IL-6 and TNF α by Sandwich ELISA for the negative control, positive control and (Positive control+ VIROSCOPE®). The doses of VIROSCOPE® (40 ml VIROSCOPE® + positive control), (20 ml VIROSCOPE® + positive control), and (5 ml VIROSCOPE® + positive control) were tested, and each sample was tested four times.

3. Results

3.1 Evaluation of VIROSCOPE® cytotoxicity

The cytotoxicity of VIROSCOPE® was dose-dependent and the cytotoxicity decreased with decreasing doses. Thus, according to NF EN ISO 10993-5, a VIROSCOPE® dose of 40 ml had mild cytotoxicity (class 2 product), whereas the doses of 20 ml and 5 ml had both slight cytotoxicity (class 1 product) (Table 1 and Figure 1).

Table 1: Results of the evaluation of VIROSCOPE® Cytotoxicity

Test	Negative control (%)	Positive control (%)	VIROSCOPE® (%)		
			Dose of 40 ml	Dose of 20 ml	Dose of 5 ml
Test Nº1	3.31	88.87	30.42	23.60	20.63
Test Nº2	3.00	81.36	11.80	7.92	6.79
Mean	3.16	85.12	21.11	15.76	13.71

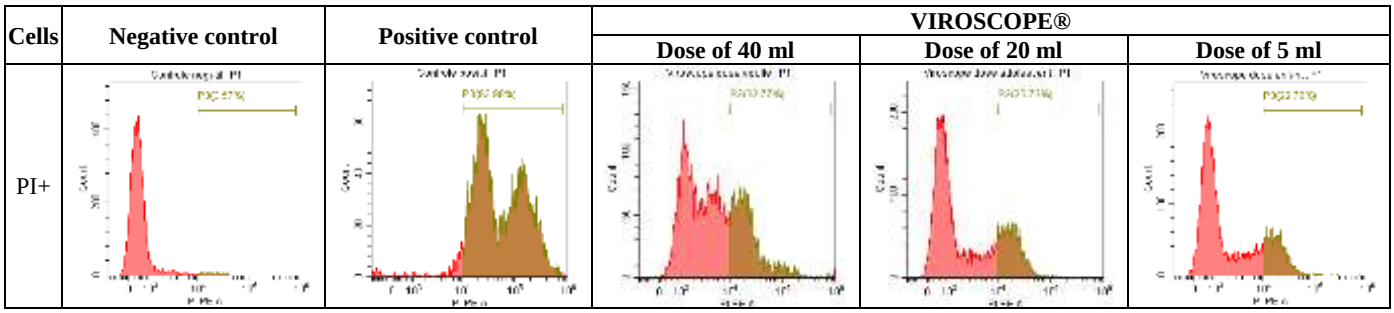

Fig 1: Illustration of cytotoxicity assessment. PI, propidium iodide

3.2 Evaluation of immune cell proliferation

The proliferation of immune cells induced by VIROSCOPE® was dose-dependent. Indeed, the 40 ml dose induced excellent proliferation of immune cells, even exceeding the results obtained for the positive control. However, doses of 20 ml and 5 ml did not induce any significant proliferation of immune cells (Table 2 and Figure 2).

Table 2: Results of the evaluation of immune cell proliferation

PCNA-producing cells	Negative control (%)	Positive control (%)	VIROSCOPE® (%)		
			Dose of 40 ml	Dose of 20 ml	Dose of 5 ml
Test Nº1	65.86	70.57	78.34	64.63	60.31
Test Nº2	73.97	75.56	80.80	77.31	67.60
Mean	69.92	73.07	79.57	70.97	63.96

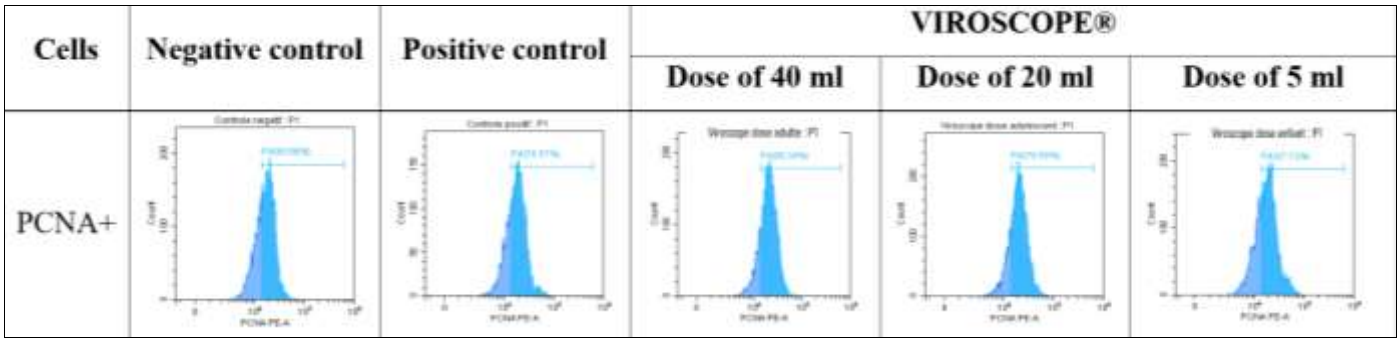

Fig 2: Illustration of immune cell proliferation assessment

3.3 Evaluation of immunostimulatory properties of VIROSCOPE®

The induction of the production of the pro-inflammatory cytokines TNF α and IL-6 by VIROSCOPE® was dose-dependent. Thus, the 40 ml dose of VIROSCOPE® did not induce a production of pro-inflammatory cytokines, whereas the doses of 20 ml and 5 ml induced low production of pro-inflammatory cytokines (Tables 3 and 4).

Table 3: Results of the evaluation of immunostimulatory properties of VIROSCOPE® (1)

Test	TNF α (pg/ml)				
	Negative control	Positive control	VIROSCOPE®		
			Dose of 40 ml	Dose of 20 ml	Dose of 5 ml
Test Nº1	28.55	798.27	0	76.60	75.50
Test Nº2	11.25	799.84	0	93.22	96.62
Test Nº3	0	789.20	0	155.53	147.57
Test Nº4	0	611.25	0	194.57	207.80
Mean	9.95	749.64	0	129.98	131.87

Table 4: Results of the evaluation of immunostimulatory properties of VIROSCOPE® (2)

Test	IL-6 (pg/ml)				
	Negative control	Positive control	VIROSCOPE®		
			Dose of 40 ml	Dose of 20 ml	Dose of 5 ml
Test Nº1	11.11	1285.78	0	14.29	15.43
Test Nº2	9.23	1300.20	0	5.02	55.14
Test Nº3	13.81	1218.74	5.56	9.99	11.09
Test Nº4	15.50	899.98	9.07	6.43	85.38
Mean	12.41	1176.17	3.66	8.93	41.76

3.4 Evaluation of the immunosuppressive properties of VIROSCOPE®

The immunosuppressive (anti-inflammatory) activity of VIROSCOPE® was dose-dependent. Thus, the 40 ml dose of VIROSCOPE® suppressed the production of TNF α and significantly reduced the production of IL-6 in an inflammatory environment. Doses of 20 ml and 5 ml induced only a very slight suppression of the production of the pro-inflammatory cytokines TNF α and IL-6 (Tables 5 and 6).

Table 5: Results of the evaluation of the immunosuppressive properties of VIROSCOPE® (1)

Test	TNF α (pg/ml)				
	Negative control	Positive control (S)	S + VIROSCOPE®		
			Dose of 40 ml	Dose of 20 ml	Dose of 5 ml
Test Nº1	28.55	798.27	0	795.81	833.84
Test Nº2	11.25	799.84	0	729.91	729.18
Test Nº3	0	789.20	0	587.97	593.32
Test Nº4	0	611.25	0	591.40	605.29
Mean	9.95	749.64	0	676.27	690.41

Table 6: Results of the evaluation of the immunosuppressive properties of VIROSCOPE® (2)

Test	IL-6 (pg/ml)				
	Negative control	Positive control (S)	S + VIROSCOPE®		
			Dose of 40 ml	Dose of 20 ml	Dose of 5 ml
Test Nº1	11.11	1285.78	58.03	1218.54	1288.40
Test Nº2	9.23	1300.20	67.77	1241.85	1269.60
Test Nº3	13.81	1218.74	271.21	1129.91	1080.49
Test Nº4	15.50	899.98	268.72	1071.84	1216.89
Mean	12.41	1176.17	166.44	1165.53	1213.85

4. Discussions

This study aimed to evaluate the *in vitro* cytotoxicity, immune cell proliferation, immunostimulatory and immunosuppressive properties of the phytomedicine "VIROSCOPE®" produced by Viroscope SARL-U in Lomé, Togo.

According to the classification criteria of the NF EN ISO 10993-5, VIROSCOPE® would have mild toxicity at a dose of 40 ml twice a day (morning and evening), and slight cytotoxicity at Dose of 20 ml and 5 ml twice a day (morning and evening). This is encouraging and ensures the safe consumption of the phytomedicine VIROSCOPE®.

According to the results obtained, VIROSCOPE® (40 ml of dose) induced excellent proliferation of immune cells. Therefore, VIROSCOPE® could be a good alternative for the prevention and treatment of immunodepression. This is in agreement with the active ingredients found in VIROSCOPE®. Indeed, the phytomedicine VIROSCOPE® contains saponosides, which are known for their immune cell proliferation properties [21–24]. However, the use of 20 ml and 5 ml produced very low immune cell proliferation. Therefore, it is imperative that immune depressed patients respect the dose of VIROSCOPE® of 40 ml twice a day (morning and evening) for optimum immune cell proliferation.

The immunosuppressive properties of VIROSCOPE® were evaluated by measuring the production of pro-inflammatory cytokines IL-6 and TNF α in an inflammatory environment. VIROSCOPE® significantly reduced the production of the pro-inflammatory cytokines IL-6 and TNF α. The phytomedicine VIROSCOPE® would be an excellent candidate for treating diseases associated with acute and chronic inflammation, and could be useful to quench cytokine storms often observed in diseases such as COVID-19 and influenza. This is consistent with the active ingredients found in VIROSCOPE®. Indeed, rutin and gallic acid, abundantly found in VIROSCOPE®, have been reported as powerful anti-inflammatory agents [25–29]. In addition, the anti-inflammatory activity of VIROSCOPE® was dose dependent. Therefore, it is necessary to maintain the dose of 40 ml twice daily for optimal anti-inflammatory activity.

In addition to its anti-inflammatory activity, VIROSCOPE® also induces a low immunostimulatory activity at dose of 20 ml and 5ml by inducing the production of the pro-inflammatory cytokines IL-6 and TNF α. Therefore, VIROSCOPE® would have dose-dependent immunomodulatory properties and the dose of VIROSCOPE® to drink will depend on the desired effect. Rutin and gallic acid (two major active ingredients found in VIROSCOPE®) have already been reported to have immunomodulatory activity [26, 30, 31].

5. Conclusion

VIROSCOPE®, a phytomedicine produced by Viroscope SARL-U, Lomé, Togo, demonstrated slight and mild cytotoxicity, excellent *in vitro* immune cell proliferation, and excellent anti-inflammatory activity. All of these activities were dose-dependent, calling for strict compliance with the dosage. Further *in vitro* studies, animal models, and clinical trials are needed to elucidate all the therapeutic activities of VIROSCOPE® and improve its formulation.

6. Perspectives

Two perspectives emerged at the end of this study.

- Evaluate the anti-diabetic, anti-oxidant, anti-microbial, and anti-cancer properties of VIROSCOPE®. Infact, as rutin and gallic acid (the major active ingredients of VIROSCOPE®) are known to have anti-diabetic, anti-oxidant, anti-microbial, and anti-cancer therapeutic activities, it would be interesting to evaluate the effects of VIROSCOPE® on diabetes, multidrug-resistant bacteria, and cancer processes.
- Clinical trials for immune cell proliferation and anti-inflammatory properties of VIROSCOPE®.

7. Conflict of interests

The authors declare that they have no conflicts of interest.

8. Funding

This study received no funding.

9. References

1. Tilburt JC, Kaptchuk TJ. Herbal medicine research and global health : An ethical analysis. Bulletin of the World Health Organization. 2008;86(8):594-599. https://doi.org/10.2471/blt.07.042820
2. Petrovska BB. Historical review of medicinal plants' usage. Pharmacognosy Reviews. 2012;6(11):1-5. https://doi.org/10.4103/0973-7847.95849
3. Šantić Ž, Pravdić N, Bevanda M, Galić K. The historical use of medicinal plants in traditional and scientific

medicine. Psychiatria Danubina. 2017, 29 Suppl 4(Suppl 4):787-792.
4. Rahman MM, Rahaman MS, Islam MR, Hossain ME, Mannan Mithi F, Ahmed M, *et al*. Multifunctional Therapeutic Potential of Phytocomplexes and Natural Extracts for Antimicrobial Properties. Antibiotics (Basel, Switzerland). 2021;10(9):1076. https://doi.org/10.3390/antibiotics10091076
5. Carradori S, Cairone F, Garzol S, Fabrizi G, Iazzetti A, Giusti AM, *et al*. Phytocomplex Characterization and Biological Evaluation of Powdered Fruits and Leaves from Elaeagnus angustifolia. Molecules. 2020;25(9):2021. https://doi.org/10.3390/molecules25092021
6. Tang Y, Liu J, Zhang D, Xu Z, Ji J, Wen C. Cytokine Storm in COVID-19 : The Current Evidence and Treatment Strategies. Frontiers in Immunology. 2020;11:1708. https://doi.org/10.3389/fimmu.2020.01708
7. Hojyo S, Uchida M, Tanaka K, Hasebe R, Tanaka Y, Murakami M, *et al*. How COVID-19 induces cytokine storm with high mortality. Inflammation and Regeneration. 2020;40(1):37. https://doi.org/10.1186/s41232-020-00146-3
8. Jiang H, Zhang Z. Immune response in influenza virus infection and modulation of immune injury by viral neuraminidase. Virology Journal. 2023;20(1):193. https://doi.org/10.1186/s12985-023-02164-2
9. Liu Q, Zhou Y, Yang Z. The cytokine storm of severe influenza and development of immunomodulatory therapy. Cellular and Molecular Immunology. 2016;13(1):3-10. https://doi.org/10.1038/cmi.2015.74
10. Zhang J-M, An J. Cytokines, inflammation, and pain. International Anesthesiology Clinics. 2007;45(2):27-37. https://doi.org/10.1097/AIA.0b013e318034194e
11. Nisar A, Jagtap S, Vyavahare S, Deshpande M, Harsulkar A, Ranjekar P, *et al*. Phytochemicals in the treatment of inflammation-associated diseases : The journey from preclinical trials to clinical practice. Frontiers in Pharmacology, 2023, 14. https://www.frontiersin.org/articles/10.3389/fphar.2023.1177050
12. Ghasemian M, Owlia S, Owlia MB. Review of Anti-Inflammatory Herbal Medicines. Advances in Pharmacological Sciences. 2016;9130979. https://doi.org/10.1155/2016/9130979
13. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017;9(6):7204-7218. https://doi.org/10.18632/oncotarget.23208
14. Fioranelli M, Roccia MG, Flavin D, Cota L. Regulation of Inflammatory Reaction in Health and Disease. International Journal of Molecular Sciences. 2021;22(10):Article 10. https://doi.org/10.3390/ijms22105277
15. Desforges JP, Sonne C, Dietz R, Levin M. Chapter 12—Immunotoxic Effects of Environmental Pollutants in Marine Mammals. In M. C. Fossi & C. Panti (Éds.), Marine Mammal Ecotoxicology. 2018, 321-343. Academic Press. https://doi.org/10.1016/B978-0-12-812144-3.00012-7
16. Bagwe SM, Kale PP, Bhatt LK, Prabhavalkar KS. Herbal approach in the treatment of pancytopenia. Journal of Complementary and Integrative Medicine. 2017;14(1). https://doi.org/10.1515/jcim-2016-0053
17. Hasan N, Sorkhy M. Herbs that promote cell proliferation. International Journal of Herbal Medicine. 2014;1:18-21.
18. Wang H, Chen Y, Wang L, Liu Q, Yang S, Wang C. Advancing herbal medicine : Enhancing product quality and safety through robust quality control practices. Frontiers in Pharmacology. 2023;14. https://www.frontiersin.org/articles/10.3389/fphar.2023.1265178
19. Balekundri A, Mannur V. Quality control of the traditional herbs and herbal products : A review. Future Journal of Pharmaceutical Sciences. 2020;6(1):67. https://doi.org/10.1186/s43094-020-00091-5
20. Muyumba NW, Mutombo SC, Sheridan H, Nachtergael A, Duez P. Quality control of herbal drugs and preparations : The methods of analysis, their relevance and applications. Talanta Open. 2021;4:100070. https://doi.org/10.1016/j.talo.2021.100070
21. Khumalo M, Qwebani-Ogunleye T, Ejidike I, Mtunzi F, Pinkoane M. Evaluation of immune booster formulation by traditional health practitioners : Phytochemical, antioxidant and mineral elements studies. International Journal of Pharma and Bio Sciences. 2018;9. https://doi.org/10.22376/ijpbs.2018.9.2.p29-37
22. Sharma R, Palanisamy A, Dhama K, Mal G, Singh B, Singh KP. Exploring the possible use of saponin adjuvants in COVID-19 vaccine. Human Vaccines & Immunotherapeutics. 2020;16(12):2944-2953. https://doi.org/10.1080/21645515.2020.1833579
23. Rajput ZI, Hu S, Xiao C, Arijo AG. Adjuvant effects of saponins on animal immune responses. Journal of Zhejiang University Science B. 2007;8(3):153-161. https://doi.org/10.1631/jzus.2007.B0153
24. Wang P. Natural and Synthetic Saponins as Vaccine Adjuvants. *Vaccines*. 2021;9(3):222. https://doi.org/10.3390/vaccines9030222
25. Ganeshpurkar A, Saluja AK. The Pharmacological Potential of Rutin. Saudi Pharmaceutical Journal : SPJ. 2017;25(2):149-164. https://doi.org/10.1016/j.jsps.2016.04.025
26. Shruthi S, Vijayalaxmi KK, Shenoy KB. Immunomodulatory Effects of Gallic Acid against Cyclophosphamide- and Cisplatin-induced Immunosuppression in Swiss Albino Mice. Indian Journal of Pharmaceutical Sciences. 2018;80(1):150-160. https://doi.org/10.4172/pharmaceutical-sciences.1000340
27. Hamada S, Kataoka T, Woo J-T, Yamada A, Yoshida T, Nishimura T, *et al*. Immunosuppressive Effects of Gallic Acid and Chebulagic Acid on CTL-Mediated Cytotoxicity. Biological & Pharmaceutical Bulletin. 1997;20(9):1017-1019. https://doi.org/10.1248/bpb.20.1017
28. Gurkan G, Erdogan MA, Yigitturk G, Erbas O. The Restorative Effect of Gallic Acid on the Experimental Sciatic Nerve Damage Model. Journal of Korean Neurosurgical Society. 2021;64(6):873-881. https://doi.org/10.3340/jkns.2021.0078
29. Kahkeshani N, Farzaei F, Fotouhi M, Alavi SS, Bahramsoltani R, Naseri R, *et al*. Pharmacological effects of gallic acid in health and diseases : A mechanistic review. Iranian Journal of Basic Medical Sciences. 2019;22(3):225-237. https://doi.org/10.22038/ijbms.2019.32806.7897
30. Ganeshpurkar A, Saluja A. In silico interaction of rutin with some immunomodulatory targets : A docking analysis. Indian Journal of Biochemistry & Biophysics.

2018, 55.
31. Mektrirat R, Chuammitri P, Navathong D, Khumma T, Srithanasuwan A, Suriyasathaporn W. Exploring the potential immunomodulatory effects of gallic acid on milk phagocytes in bovine mastitis caused by *Staphylococcus aureus*. Frontiers in Veterinary Science. 2023, 10. https://www.frontiersin.org/articles/10.3389/fvets.2023.1255058