FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 4 No. 4, December, 2020, pp 108 – 115
DOI: https://doi.org/10.33003/fjs-2020-0404-445

IMMUNOSTIMULATORY ACTIVITY OF AQUEOUS LEAVES EXTRACT OF *CASSIA OCCIDENTALIS* ON INNATE AND ADAPTIVE IMMUNE RESPONSE IN MICE

[1]Hamid, K.M., [1]Kalgo, M.U., [1]Isyaku, A., [1]Abdullahi, H., [1]Usman, A.B., [1]Alkali, S., [1]Tijjani, M., [1]Shehu, A.A., [1] Umahi, N.P.F., *[2]Aliyu, M.

[1] Department of Immunology, School of Medical Laboratory Sciences, Usmanu Danfodiyo University Sokoto, Nigeria
[2] Department of Medical Microbiology and Parasitology, Faculty of Clinical Sciences, College of Health Sciences, Bayero University. P.M.B. 3011, Kano, Nigeria.

***Corresponding Author: maliyu.med@buk.edu.ng, +2348038468613**

ABSTRACT

Different types of plants have been studied for their roles in immunostimulation and treatment of other diseases. The study aimed at evaluating the stimulatory activity of aqueous leaf extract of *Cassia occidentalis* on innate and adaptive immune responses in mice. Study mice were grouped into five and studied for 21 days; group I (control group) received normal saline. While the treatment groups: group II - V received aqueous leaf extract of *C. occidentalis* at different concentrations (250, 500, 1000, and 1500) mg/kg body weight (BW). The mice were sensitized and challenged with sheep red blood cells (SRBCs) on day 13th and 18th of the treatment, respectively. On the 19th, 20^{th,} and 21st days, the animals' delayed-type hypersensitivity (DTH) responses in each group were measured using a digital Vernier calliper, and humoral immune response was evaluated using the haemagglutination (HA) antibody technique. Blood samples were collected for Carbonic particle clearance index (K) and phagocytic index of Macrophage (α). Organ weight index (g/100g) was also recorded (α). The result showed a significant increase in the Carbonic particle clearance index ($p=0.02$) and phagocytic index of Macrophage ($p<0.0001$) in a dose-dependent manner. The study revealed a statistically significant difference in HA titres across the five groups, ($p=0.02$), also the DTH shows a statistically significant difference across the time (24 hours, 48 hours, 72 hours), Wilks' Lambda = 0. 291, F = 15. 86, p = 0. 0001. The aqueous leaf extract of *C. occidentalis* has an immunostimulatory effect on innate and adaptive immunity.

Keywords: *Cassia occidentalis*, innate immunity, adaptive immunity, immunostimulation, murine

INTRODUCTION

Plants have been in use for long as medicine in African subcontinents and have been an essential source of drugs, particularly in traditional medicine (Bako *et al.*, 2005; Sumalatha *et al.*, 2012). Immunomodulatory therapy could provide an alternative to conventional chemotherapy for various diseased conditions, especially when the host's defense mechanisms have to be activated under the conditions of impaired immune responsiveness (Singh *et al.*, 2015). Some plants have medicinal properties since the early stages of the development of medicine; the constant search for the medicinal plant has identified several types that have contributed to the treatment of various diseases (Sumalatha *et al.*, 2012).

C. occidentalis is a medicinal herb commonly used in treating bacterial and fungal diseases (Ambasta, 1986). It is extensively used in the indigenous and folklore medicine system as an antidote of poisons, blood purifier, expectorant, anti-inflammatory agent, and a remedy for the treatment of liver diseases (Emmanuel *et al.*, 2010). It is one of such frequently used African medical plants; its species are found in large number all over regions (Saidu *et al.*, 2011), and that has long been known among the natives of the coast of West Africa, particularly in Nigeria and Ghana to be effective in the treatment of dysentery and diarrhea which are mainly caused by micro-organisms (Omoregbe *et al.*, 1996).

The *C. occidentalis* roots, flowers, seeds, and leaves have been employed in herbal medicine worldwide for a variety of purposes such as purgatives, analgesic (Sini *et al.*, 2010), and wound healing (Garba *et al.*, 2015). The leaf extract possesses a considerable number of medicinal properties, it was found to have a diuretic and antioxidant effect (Ntchapda *et al.*, 2015), effective for relief of dysmenorrhoea (Andel *et al.*, 2014), antibacterial and antitubercular activity (Yeragamreddy *et al.*, 2013), improves anaemia from other diseases (Ibrahim *et al.*, 2010), detoxification and liver protection (Yadav *et al.*, 2010), and treatment of fevers (Singh *et al.*, 2020). As reviewed by Verma *et al.* (2010), "The plant is also used to cure sore eyes, haematuria, rheumatism, typhoid, asthma, and haemoglobin disorders and was also reported to cure leprosy." It is

commonly employed as a topical agent to heal skin conditions (Caseres *et al.*, 1993; Ogunkunle *et al.*, 2006).

Immune regulation is vital in maintaining normal immunity, and the search for herbal immunomodulatory compounds to treat various infections by enhancing the body's natural resistance is of growing interest (Brindha, 2016). Immunomodulatory compounds derived from medicinal plants may be free from toxic effects; it could be safe and free from any undesirable side effect (Laemmli, 1970).

The concept of immunomodulation relates to non-specific activation of the immune system, which primarily implies a non-antigen dependent stimulation of function and efficiency of macrophages, granulocytes, complement, Natural Killer (NK) cells, lymphocytes, and also the production of various effector molecules by activated cells (Sumalatha *et al.*, 2012). Medicinal plants need to be evaluated, given proper recognition, and developed to improve their efficacy, safety, availability, and vast application at low cost (Tilburt *et al.*, 2008).

There is a paucity of data on the stimulatory activity of aqueous leaf extract of *C. occidentalis* on innate, humoral immune, and delayed-type hypersensitivity (DTH) responses. Our study aimed to provide vital information on the effects of aqueous leaf extract of *C. occidentalis* on macrophages' phagocytic function, humoral immune response against SRBCs and DTH response in mice.

MATERIALS AND METHODS

Plant Collection and Identification

Fresh leaves of *C. occidentalis* were collected locally. The plant taxonomic identification was carried out at the Herbarium section of Botany Unit, Department of Biological Sciences, Faculty of Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The voucher number issued was UDUS/ANS/0110.

Preparation and Extraction

The aqueous extract of *C. occidentalis* was collected from the Department of Immunology, School of Medical Laboratory Sciences which was initially stored and preserved at 5°C in airtight bottles and was labelled as *C. occidentalis* aqueous extracts.

Experimental Animals

A total of 20 mice of 8-12 weeks old, weight between 17-38 g of either sex (9 Females; 11 Males) were purchased from the Department of Veterinary Pharmacology, Faculty of Veterinary
Medicine, Ahmadu Bello University (ABU), Zaria, and kept in the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto (UDUS), Nigeria. They were allowed to acclimatize for two weeks and fed with standard pelletized growers' feed (Vital feed, Jos, Plateau) and water *ad libitum.* The Animal Ethics Committee of Faculty of Pharmaceutical Sciences, UDUS, approved the study (PTAC/Co/(AE)/OT/13-19). The experiments were conducted following regulations governing animal research.

Animal grouping and treatment

The animals were randomly divided into five groups of four each. Group I received 10ml/kg BW of normal saline, and Group II-V (treatment groups) received different concentrations of *C. occidentalis* (i.e., 250, 500, 1000, 1500 mg/Kg BW) respectively, orally for 21 days. At the end of the experiment all the mice survived.

Carbon Clearance Assay

A carbon clearance assay was used to assess the effect of the extract treatment on the Macrophage as previously described (Yin *et al.*, 2016). Indian ink (Camel brand) suspension was diluted in a ratio of 1:50 with normal saline and used for a carbon clearance test in a dose of 0.1 ml/mice. One hour after the last treatment, the animals were weighed, then injected with 0.1 ml of Indian ink via the tail vein and timed immediately. After the ink injection, at the time of 1 min (T1) and 15 min (T2).
Blood samples were collected from orbital venous plexus using heparinized microhematocrit capillary tubes. The blood was immediately added with 3 ml 0.1% Na_2CO_3 solution to lyses the erythrocytes, and the absorbance of the mixture was measured at 675 nm using a spectrophotometer. The absorbances were A1 (at the time of T1) and A2 (at the time of T2), respectively, using the Na_2CO_3 solution's absorbance as a blank control. Then mice were euthanatized under anesthesia. The liver and spleen were exercised from the animal and were weighed immediately. The carbonic particle clearances index (K) and macrophage phagocytose index (α), as well as organ weight index, were calculated as below:

$$\text{Carbonic particle clearances index (K)} = \log A1 - \log A2$$

$$\text{Macrophage phagocytose index } (\alpha) = \frac{K^{1/3} \times \text{weight}}{\text{Liver + spleen weight}}$$

$$\text{Index (g/100g)} = \frac{\text{Weight of spleen}}{\text{Body weight} \times 10}$$

Hemagglutination assay

Preparation of SRBCs (Antigen)

SRBCs were prepared as described by Dash *et al.* (2006). Briefly, fresh blood sheep were collected from a local slaughterhouse in freshly prepared sterile Alsever's solution in a 1:1 ratio. Then centrifuged at 2000 rpm for 10 minutes and washed with physiological saline 4-5 times. The cells were suspended in buffered saline and finally adjusted to a 1×10^8 cells/ml concentration for sensitization and challenge.

Antibody response to SRBCs

On the 13th and the 18th day of the study, the mice from all the groups were sensitized and challenged, respectively, with SRBCs in normal saline (0.1 ml of suspension containing 1×10^8 SRBCs), intraperitoneally. Blood was withdrawn (24 hours after the last dose) from the retro-orbital plexus of all antigenically sensitized and challenged mice, then centrifuged at 3000 rpm for 5 minutes to obtain serum.

Direct Hemagglutination

The procedure was described by Haghighi *et al.*, (2005). Briefly, the serum sample was incubated at 56 °C for 30 min to inactivate the complement. Fifty microliters (50 µl) of Phosphate Buffered Saline (PBS) containing 0.05% Bovine Serum Albumin was dispensed into each well of round-bottomed 96- well microplate. Serum samples (50 µl) were then added and serially double diluted in the wells from columns 2 to 12. The first column (PBS only) of wells was considered as blank. Then, 50 µl of 1% SRBCs in PBS was added to all the wells to make a 100 µl final volume. Subsequently, the plates were shaken for 1 min and incubated for 24 hours at 37℃ and agglutination was monitored visually. The highest dilution capable of visible agglutination was considered as the antibody titre. A positive result was recorded when at least 50% of SRBCs agglutination was observed.

Delayed type hypersensitivity reaction

On day 13, the mice were subcutaneously injected with 0.1 ml of SRBCs suspension containing 1 x 10^8 cells/ml on the right hind footpad, while the left hind footpad received 0.1 ml of normal saline only. On day 18, the right hind footpad received 0.1 ml of normal saline only (i.e., control foot). Whereas left hind footpad was challenged subcutaneously with 0.1 ml of 1 x 10^8 cells /ml SRBCs suspension (i.e., challenge foot). The thickness of the left and right hind footpads were measured at 24, 48, and 72 hours using RD DC 706 digital Vernier callipers (Raider Pro Professional Tools Co. Ltd., China). The difference in the thickness of the right hind footpad and the left hind footpad was used to measure DTH response (Doherty, 1981; Smith *et al.*, 2010).

Statistical Analysis

The results obtained were entered into SPSS version 25 for analysis. Continuous variables were expressed as mean and standard deviation (SD) or Median. Test for normality was performed to ascertain the normal distribution of the variables. Macrophage phagocytose index and DTH were normally distributed, while Carbonic particle clearance index, Organ weight index and HA titre were not normally distributed based on tests of normality results: Shapiro-Wilk, supported by Q-Q plot. Therefore a one-way-between- groups ANOVA for macrophage phagocytose index, one-way repeated measures ANOVA for DTH and Kruskal Wallis test for carbonic particle clearance index, Organ weight index and HA titre was carried out to compare mean or median respectively. Bonferroni or Mann Whitney tests were used as post hoc tests to compare some groups. The p-value ≤ 0.05 was used to determine the level of statistical significance.

RESULTS

Effect on Carbonic Particle Clearance and Macrophage Phagocytose Indexes

As depicted in table 1, Group V recorded the highest K median scores (Md= 0.0190) while the least is group I (Md= 0.0001). A Kruskal-Wallis test revealed a statistically significant difference in K across the five groups (χ^2 =11.52, p=0.02). A Post-hoc test using the Mann-Whitney U test revealed a significant difference in K between Group I vs. Group IV (p=0.04), and Group I vs. Group V (p=0.04). Again, on macrophage phagocytose index, Group V recorded the highest mean scores (M: 1.87, SD: 0.12) while the least is a group I (M: 0.37, SD: 0.03). There was a statistically significant difference in Macrophage phagocytose index across the groups after treatment with *C. occidentalis* (p<0.0001). Post-hoc comparisons indicated that the macrophage phagocytoses index mean score for normal control was significantly different from group II (p<0.0001), group III (p=0.005), group IV (p<0.0001), and group V (p<0.0001).

Table 1: Effect of aqueous leaf extract of *C. occidentalis* on Carbon clearance and Macrophage Phagocytose indexes in Mice

Groups (N=20)	Dose (kg BW)	Carbon clearance index		Macrophage Phagocytose index
		Median	χ^2	Mean (SD)
I (Normal saline)	10 ml	0.0001 ^{c*,d*}	11.52	0.37 (0.03) ^{a***,b**,c***,d***}
II (*C. occidentalis*)	250 mg	0.0002		0.40 (0.09)
III(*C. occidentalis*)	500 mg	0.0002		0.87 (0.19)
IV(*C. occidentalis*)	1000 mg	0.0104		1.26 (0.10)
V (*C. occidentalis*)	1500 mg	0.0190		1.87 (0.12)
p-value		0.02		<0.0001

a= grp I vs II, b= grp I vs III, c= grp I vs IV, d= grp I vs V, *p<0.05, **p<0.006, ***p<0.0001

Effect on Organ Weight Index

From table 2, Group V recorded the highest organ weight index median score (Md= 0.0410) while the least is group I (Md= 0.0001). A Kruskal-Wallis test revealed a statistically significant difference in organ weight index (g/100 g) across the five groups (χ^2 = 12.63, p=0.01). A Post-hoc test using the Mann-Whitney U test revealed a significant difference in organ weight index between Group I vs. Group IV (*p*=0.04) and Group I vs. Group V (p=0.04).

Table 2: Effect of aqueous leaf extract of *C. occidentalis* on organ weight Index in Mice

Group N=20	**Dose (Kg BW)**	**Organ weight index**		**p-value**
		Median	χ^2	
I (Normal saline)	10ml	0.0001 [a*, b*]	12.63	0.01
II (*C. occidentalis*)	250 mg	0.0020		
III (*C. occidentalis*)	500 mg	0.0090		
IV (*C. occidentalis*)	1000 mg	0.0099		
V (*C. occidentalis*)	1500 mg	0.0410		

a= grp I vs. IV, b= grp I vs. V, *p<0.05

Effect on Humoral Immune Response against Sheep Red Blood Cells

As shown in Table 3 below, the HA titre of the Mice receiving the highest dose (group V) of the plant extract recorded a highest median score (Md = 64) while those in group I and II recorded the least median scores. A Kruskal-Wallis test revealed a statistically significant difference in HA titres across the five groups (χ^2 =12.26, p=0.02). A Post-hoc test using the Mann-Whitney U test revealed a significant difference in HA titre between Group I vs. Group III (p=0.03), Group I vs. Group IV (*p*=0.04), Group I vs. Group V (p=0.04).

Table 3: Effect of aqueous leaf extract of *C. occidentalis* on humoral immune response in mice

Groups (N=20)	**Dose (Kg BW)**	**HA titre Median**	χ^2	***p*-value**
I (Normal saline)	10ml	8 [a*, b*, c*]	12. 26	0. 02
II (*C. occidentalis)*	250 mg	8		
III *(C. occidentalis)*	500 mg	16		
IV *(C. occidentalis)*	1000 mg	32		
V (*C. occidentalis)*	1500 mg	64		

a= grp I vs III, b= grp I vs IV, c= grp I vs V, *p<0.05

Effect on Delayed-Type Hypersensitivity response

The DTH response at 72 hours recorded a highest mean difference (SD) 1.46 (0.89) whereas DTH response at 24 hours recorded the least mean difference. A one-way repeated measures ANOVA was conducted to compare the DTH response between 24 hours, 48 hours and 72 hours. There was a significant statistical difference across the time (24 hours: M= 0.33 mm, SD= 0.10), (48 hours: M= 0.44 mm, SD= 0. 11) and (72 hours: M= 1.46 mm, SD= 0.89), Wilks' Lambda = 0. 291, F = 15. 86, p = 0. 0001. A Post-hoc comparisons using the Bonferroni tests indicated that the mean DTH response at 24 hours (M = 0. 33 mm, SD = 0. 10) was significantly different from that at 48 hours (M =0. 44 mm, SD = 0.11), p = 0. 007 and that at 72 hours (M = 1. 46 mm, SD = 0. 89), p= 0. 0001. So also mean DTH response at 48 hours (M =0. 44 mm, SD = 0.11) was significantly different from that at 72 hours (M = 1. 46 mm, SD = 0. 89), p = 0. 001 (Table 4).

Table 4: Comparison of the mean difference of DTH response at 24, 48, and 72 hours

Time (hr)	**DTH (mm)**	**Wilks' Lambda test**		**Post hoc test**		**95% CI**	
	Mean difference (SD)	**Value**	***p*-value**	**Time comparison (hr)**	***p*-value**	**Lower bound**	**Upper bound**
24	0. 33(0. 10)	0. 29	0. 0001	24 vs. 48	0. 007	-0. 184	-0. 028
48	0. 44(0. 11)			24 vs. 72	0. 0001	-1. 703	-0. 551
72	1. 46(0. 89)			48 vs. 72	0. 001	-1. 582	-0. 460

DISCUSSION

Medicinal plants have been used as an alternative to orthodox medicine in many countries, Nigeria inclusive. Despite their widespread use, few scientific studies have been undertaken to ascertain the efficacy of traditional remedies (Graça *et al.*, 2017). Thousands of plant species have been identified as a good source of therapeutics, and the use of these compounds is gradually increasing (Ishii *et al.*, 1984).

In the present study, *C. occidentalis* showed a significant increase in carbonic particle clearance (K) and macrophage phagocytosis index (α) in the dose-dependent increase suggesting the *C. occidentalis* stimulatory effect on macrophages. Macrophages are professional antigen-presenting cells that phagocytose pathogens and debris as innate immunity effector cells. Moreover, macrophages process and present the antigens they recognise and engulf to $CD4^+$ helper T cells to initiate an adaptive response, thereby bridging innate and adaptive immunity (Gokhale *et al.*, 2003).

The current study findings are in line with previous studies (Anarthe *et al.*, 2014; Bin-Hafeez *et al.*, 2003; Vikasari *et al.*, 2015). The Stimulatory activity of *C. occidentalis* on immune cells may be due to the presence of various Phyto-constituents present in the extract like quinines, flavonoids, tannins, and alkaloids, which are already reported to possess immunomodulatory activity (Anarthe *et al.*, 2014). Several studies reported the immunostimulatory effect of plants: Methanolic extract of *Swietenia mahagoni* seeds (Hajra *et al.*, 2012), an aqueous extract of Tridax (Tiwari *et al.*, 2004), an aqueous extract of seeds of *Abrus precatorius Linn* (Tilwari *et al.*, 2011).

The carbon clearance assay shows the rate at which carbon particles are removed from blood after systemic injection of carbon particle in the form of an ink, and the rate of the clearance was deduced mathematically through a given equation (Gokhale *et al.*, 2003). The removal of carbon particles was made by phagocytic cells such as macrophages. At the same time, macrophage activity were assessed by the assay (Ponkshe *et al.*, 2002). The phagocytic process defect may lead to several disease conditions in humans (White *et al.*, 1986). Because macrophages are essential in the secretion of several cytokines, it ultimately leads to the activation of other immune cells (Tilwari *et al.*, 2011).

C. occidentalis showed a significant increase in organ weight index (g/100g) at higher dose. This implies the plant extract may potentially has concentration dependant effects on the organ weight. Several studies are in line with our finding, Hajra *et al.* (2012) on Methanolic extract of *Swietenia mahagoni* seeds, Anarthe *et al.* (2014) on water extract of *Stachytarpheta jamaicensis L.* in albino rats, and Tiwari *et al.* (2004) on aqueous extract of *Tridax procumbens*. However, in contrary to this study findings Vikasari *et al.* (2015) reported a significant decrease in organ weight of rats after treatment with Methanolic extract of *Trigonella foenum graecum* whole plant and also that of Tilwari *et al.* (2011), who reported that immunomodulatory activity of the aqueous extract of seeds of *Abrus precatorius Linn*.

The humoral immune response against SRBCs antigen reveals a significant increase in HA antibody titres with an increase in plant extract concentration; this implies treatment with the formulation may have the ability to activate lymphocytes, especially the B lymphocytes and other cells associated with humoral immune response Antibodies are produce by plasma cells upon the interaction between B lymphocytes and antigen thus lead to its proliferation and differentiation into the antibody secretion cells. The antibodies produced were set to bind to the antigen and neutralised or facilitated recognition by phagocytes; this leads to the ultimate elimination of the antigen (Fulzele *et al.*, 2003; Gokhale *et al.*, 2003).

Several studies reported a significant increase in circulating antibodies after treatment with different plant extract, *Aegle marmelos* (Patel *et al.*, 2010), *Acacia polyacentha wild* (Ismail *et al.*, 2009), *Adensonia digitata* (Rahul *et al.*, 2015), *Argyreia speciosa* root (Gokhale *et al.*, 2003), and *Swietenia mahagoni* (Hajra *et al.*, 2012). However, Tilwari *et al.* (2011) reported inhibitory effect on humoral immune response in mice after treatment with *Abrus precatorius.* The difference may be attributed to suppression of functional activity of antigen presenting cells or type and level of cytokines present at the microenvironment after activation of lymphocytes (Allison, 2000).

This study reported no significant difference between the normal control group and group II (a group that received a low dose of the extract). The indifference may be attributed to the extract's small dosage, which may not significantly modulate the immune response as a low dose might bring about little or no detectable immune response against SRBCs antigen between the groups. Mirabeau *et al.* (2012) reported a much higher immune-stimulating effect in the presence of a higher plant extract dose. . Of note the Lethal dose (LD_{50}) of our extract was 5000 mg/kg BW using Lorke's method (Lorke, 1983).

After 24 hours, the result on DTH did not show a significant increase, but after 48 hours, the result shows a significant increase, and a more robust increase in the thickness of the footpad was observed after 72 hours. The above suggests that the plant has immunostimulatory activities on DTH response. The increment may be attributed to the ability of the Extract to activate lymphocytes and other immune cells, thereby increasing cell-mediated immunity.

In DTH response, activated T cells activate and recruit more macrophages through secretion of lymphokines, which increases vascular permeability, induces vasodilatation, and produces inflammation (Nfambi *et al.*, 2015). Previous studies (Kumolosasi *et al.*, 2018; Manjuladevi *et al.*, 2013; Ramesh *et al.*, 2016) also reported an increase in DTH response after treatment with plant materials. Further study will be required

to include relevant control groups to improve on the credence of this study findings.

CONCLUSION

The aqueous leaf extract of *Cassia occidentalis* has immunostimulatory activities on macrophage phagocytic function, humoral and cell-mediated immune responses in a dose-dependent manner. In brief, *Cassia occidentalis* inherent ability to induce activation of lymphocytes involved in humoral and cell mediated immune responses indicates the immunomodulatory potential of the extract and in-depth research needed to ascertain our finding.

Conflict of Interests

The authors declare no conflict of interest.

Funding/Support

No funding or support available to this paper.

REFERENCES

Allison, A. C. (2000). Immunosuppressive drugs: the first 50 years and a glance forward. *Immunopharmacology*, **47**(2-3): 63-83. doi:10.1016/s0162-3109(00)00186-7

Ambasta, S. S. (1986). *The useful plants of India*: Publications & Information Directorate CSIR, New Delhi, India.

Anarthe, S. J., Sunitha, D., & Raju, M. G. (2014). Immunomodulatory activity for methanolic extract of *Trigonella foenum graecum* whole plant in wistar albino rats. *American Journal of Phytomedicine and Clinical Therapeutics*, **2**(9): 1081-1092. 1081-1092. doi=10.1.1.684.988

Andel, T., de Boer, H., Barnes, J., & Vandebroek, I. (2014). Medicinal plants used for menstrual disorders in Latin America, the Caribbean, sub-Saharan Africa, South and Southeast Asia and their uterine properties: A review. *Journal of Ethnopharmacology*, **155**: 992-1000. doi:10.1016/j.jep.2014.06.049

Bako, S., Bakfur, M., John, I., & Bala, E. (2005). Ethnomedicinal and phytochemical profile of some savanna plant species in Nigeria. *International Journal of Botany*. **1**: 147-150 doi=ijb.2005.147.150

Bin-Hafeez, B., Haque, R., Parvez, S., Pandey, S., Sayeed, I., & Raisuddin, S. (2003). Immunomodulatory effects of fenugreek (*Trigonella foenum graecum* L.) extract in mice. *International immunopharmacology*, **3**(2): 257-265. doi: 10.1016/S1567-5769(02)00292-8.

Brindha, P. (2016). Role of phytochemicals as immunomodulatory agents: A review. *International Journal of Green Pharmacy (IJGP)*, **10**(1). DOI: http://dx.doi.org/10.22377/ijgp.v10i1.600

Caseres, A., & Figueroa, L. (1993). Plants used in Guatemala for the treatment of respiratory diseases. 2. *Journal of ethnopharmacology.*, **16**: 77-82. doi: 10.1016/0378-8741(93)90053-8.

Dash, S., Nath, L., Bhise, S., Kar, P., & Bhattacharya, S. (2006). Stimulation of immune function activity by the alcoholic root extract of *Heracleum nepalense* D. Don. *Indian journal of pharmacology*, **38**(5): 336. DOI: 10.4103/0253-7613.27701

Doherty, N. (1981). Selective effects of immunosuppressive agents against the delayed hypersensitivity response and humoral response to sheep red blood cells in mice. *Agents and Actions*, **11**(3): 237-242. doi.org/10.1007/BF01967620

Emmanuel, S., Rani, M. S., & Sreekanth, M. R. (2010). Antidiabetic activity of *Cassia occidentalis* Linn. In streptozotocin-induced diabetic rats: A dose dependent study. *International Journal of Pharmacology and Bioscience*, **1**(4): 14-25. doi=10.1.1.440.2521

Fulzele, S., Satturwar, P., Joshi, S., & Dorle, A. (2003). Study of the immunomodulatory activity of *Haridradi ghrita* in rats. *Indian journal of pharmacology*, **35**(1): 51-54.

Garba, R., Saidu, A., Adeyemi, H., & Muhammad, H. (2015). Effect of methanolic extract of *Cassia occidentalis* L. root bark on body weight and selected biochemical parameters in alloxan induced diabetic rats. *British Journal of Pharmacology and Toxicology*, **6**(2): 39-49. DOI: 10.19026/bjpt.6.5189

Gokhale, A. B., Damre, A. S., & Saraf, M. N. (2003). Investigations into the immunomodulatory activity of *Argyreia speciosa*. *Journal of Ethnopharmacology*, **84**(1): 109-114. doi:10.1016/s0378-8741(02)00168-x

Graça, V. C., Barros, L., Calhelha, R. C., Dias, M. I., Ferreira, I. C., & Santos, P. (2017). Bio-guided fractionation of extracts of *Geranium robertianum* L.: Relationship between phenolic profile and biological activity. *Industrial Crops and Products*, **108**: 543-552. doi:10.1016/j.indcrop.2017.07.016

Haghighi, H. R., Gong, J., Gyles, C. L., Hayes, M. A., Sanei, B., Parvizi, P., Gisavi, H., Chambers, J. R., & Sharif, S. (2005). Modulation of antibody-mediated immune response by probiotics in chickens. *Clinical and diagnostic laboratory immunology*, **12**(12): 1387-1392. doi:10.1128/CDLI.12.12.1387-1392.2005

Hajra, S., Mehta, A., & Pandey, P. (2012). Immunostimulating activity of methanolic extract of Swietenia mahagoni seeds. *International Journal of Pharmacy and Pharmaceutical Science*, **4**(1): 442-445.

Ibrahim, M. A., Aliyu, A. B., Sallau, A. B., Bashir, M., Yunusa, I., & Umar, T. S. (2010). *Senna occidentalis* leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anemia and organ damage.

Pharmacognosy research, **2**(3): 175-180. doi:10.4103/0974-8490.65513

Ishii, R., Yoshikawa, K., Minakata, H., Komura, H., & Kada, T. (1984). Specificities of bio-antimutagens in plant kingdom. *Agricultural and Biological Chemistry*, **48**(10): 2587-2591. doi:10.1271/bbb1961.48.2587

Ismail, S., & Asad, M. (2009). Immunomodulatory activity of *Acacia catechu*. *Indian Journal of Physiology and Pharmacology*, **53**(1): 25-33.

Kumolosasi, E., Ibrahim, S. N. A., Shukri, S. M. A., & Ahmad, W. (2018). Immunostimulant activity of standardised extracts of *Mangifera indica* leaf and *Curcuma domestica* rhizome in mice. *Tropical Journal of Pharmaceutical Research*, **17**(1): 77-84. DOI: 10.4314/tjpr.v17i1.12

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *nature*, **227**(5259): 680-685. doi.org/10.1038/227680a0

Lorke, D. (1983). A new approach to practical acute toxicity testing. *Archives of toxicology*, **54**(4): 275-287. doi.org/10.1007/BF01234480.

Manjuladevi, K., Reddy, G., Kothai, A., Thenmozhi, M., Dhanalakshmi, M., & Sarumathy, S. (2013). Evaluation of immunomodulatory activity of aqueous extract of a polyherbal formulation by in vivo method. *Asian Journal of Pharmaceutical and Clinical Research*, **6**: 129-133.

Mirabeau, T. Y., & Samson, E. S. (2012). Effect of *Allium cepa* and *Allium sativum* on some immunological cells in rats. *African Journal of Traditional, Complementary and Alternative Medicines*, **9**(3): 374-379.

Nfambi, J., Bbosa, G. S., Sembajwe, L. F., Gakunga, J., & Kasolo, J. N. (2015). Immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in Wistar albino rats. *Journal of Basic & Clinical Physiology & Pharmacology*, **26**(6): 603-611. doi:10.1515/jbcpp-2014-0104

Ntchapda, F., Barama, J., Kemeta Azambou, D. R., Etet, P. F., & Dimo, T. (2015). Diuretic and antioxidant activities of the aqueous extract of leaves of *Cassia occidentalis* (Linn.) in rats. *Asian Pacific Journal of Tropical Medicine*, **8**(9): 685-693. doi:10.1016/j.apjtm.2015.07.030

Ogunkunle, A., & Ladejobi, T. A. (2006). Ethnobotanical and phytochemical studies on some species of Senna in Nigeria. *African Journal of Biotechnology*, **5**(21).

Omoregbe, R., Ikuebe, O., & Ihimire, I. (1996). Antimicrobial activity of some medicinal plants extracts on *Escherichia coli*, *Salmonella paratyphi* and *Shigella dysenteriae*. *African Journal of Medicine and Medical Sciences*, **25**(4): 373.

Patel, P., & Asdaq, S. M. B. (2010). Immunomodulatory activity of methanolic fruit extract of *Aegle marmelos* in experimental animals. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*, **18**(3): 161-165. doi:10.1016/j.jsps.2010.05.006

Ponkshe, C., & Indap, M. M. (2002). In vivo and in vitro evaluation for immunomodulatory activity of three marine animal extracts with reference to phagocytosis. *Indian Journal of Experimental Biology*, 40(12):1399-402.

Rahul, J., Jain, M. K., Singh, S. P., Kamal, R. K., Naz, A., Gupta, A. K., & Mrityunjay, S. K. (2015). *Adansonia digitata* L.(baobab): a review of traditional information and taxonomic description. *Asian Pacific Journal of Tropical Biomedicine*, **5**(1): 79-84.

Ramesh, K., Kambimath, R. S., & Venkatesan, N. (2016). Study of immunomodulatory activity of aqueous extract of *Carica papaya* in Wistar rats. *National Journal of Physiology, Pharmacy and Pharmacology*, **6**(5): 442-444. doi: 10.5455/njppp.2016.6.0512331052016

Saidu, T., & Abdullahi, M. (2011). Phytochemical determinations and antibacterial activities of the leaf extracts of *Combretum molle* and *Gossypium arboretum*. *Bayero Journal of Pure and Applied Sciences*, **4**(2): 132-136.

Singh, H., Chahal, P., Mishra, A., & Mishra, A. K. (2020). An up-to-date review on chemistry and biological activities of *Senna occidentalis* (L.) Link Family: Leguminosae. *Advances in Traditional Medicine*, **20**(3): 263-278. doi:10.1007/s13596-019-00391-z

Singh, V., Dwivedi, P., Chaudhary, B., & Singh, R. (2015). Immunomodulatory effect of *Gymnema sylvestre* (R. Br.) leaf extract: an in vitro study in Rat model. *PloS one*, **10**(10): e0139631. doi.org/10.1371/journal.pone.0139631

Sini, K., Karpakavalli, M., & Sangeetha, P. (2010). Analgesic and antipyretic activity of *Cassia occidentalis Linn*. *World Applied Sciences Journal*, **11**(10): 1216-1219.

Smith, M., & White, K. (2010). Establishment and comparison of delayed-type hypersensitivity models in the B6C3F1 mouse. *Journal of immunotoxicology*, **7**: 308-317. doi:10.3109/1547691X.2010.509707

Sumalatha, R. B. P., Ballal, S. R., & Acharya, S. (2012). Studies on immunomodulatory effects of *Salacia chinensis* L. on albino rats. *Journal of Applied Pharmaceutical Sciences*, **2**: 98-107.

Tilburt, J. C., & Kaptchuk, T. J. (2008). Herbal medicine research and global health: an ethical analysis. *Bulletin of the World Health Organization*, **86**: 594-599.

Tilwari, A., Shukla, N. P., & Pathirissery, U. D. (2011). Immunomodulatory activity of the aqueous extract of seeds of *Abrus precatorius Linn* (Jequirity) in mice. *Iranian Journal of Immunology*, **8**(2): 96-103.

Tiwari, U., Rastogi, B., Singh, P., Saraf, D. K., & Vyas, S. P. (2004). Immunomodulatory effects of aqueous extract of *Tridax procumbens* in experimental animals. *Journal of Ethnopharmacology*, **92**(1): 113-119. doi: 10.1016/j.jep.2004.02.001.

Verma, L., Khatri, A., Kaushik, B., Patil, U. K., & Pawar, R. S. (2010). Antidiabetic activity of *Cassia occidentalis* (Linn) in normal and alloxan-induced diabetic rats. *Indian journal of pharmacology*, **42**(4): 224-228. doi:10.4103/0253-7613.68422

Vikasari, S., Soemardji, A., Sutjiatmo, A., & Yani, S. (2015). Immunomodulatory Effect of Water Extract of Stachytarpheta jamaicensis (L.) Vahl ARTICLE INFO ABSTRACT. *Journal of Applied Pharmaceutical Science*, **5**: 62-66. doi:10.7324/JAPS.2015.58.S10

White, C. J., & Gallin, J. I. (1986). Phagocyte defects. *Clinical immunology and immunopathology*, **40**(1): 50-61.

Yadav, J., Arya, V., Yadav, S., Panghal, M., Kumar, S., & Dhankhar, S. (2010). *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*, **81**(4): 223-230.

Yeragamreddy, P., Peraman, R., Routhu, H., & Babu, N. (2013). In vitro Antitubercular and Antibacterial activities of isolated constituents and column fractions from leaves of *Cassia occidentalis*, *Camellia sinensis* and *Ananas comosus*. *African Journal of Pharmacology and Therapeutic*, **2**: 116-123.

Yin, J.-j., Zhou, Q., Wang, L., Xu, W., & Zhang, J.-l. (2016). Protective effect of extract of *Mauremys mutica* against cyclophosphamide (CY)-induced suppression of immune function in mice. *Food and Agricultural Immunology*, **27**(4): 577-588. doi.org/10.1080/09540105.2016.1148122