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PHYTOCHEMICAL SCREENING, ACUTE TOXICITY STUDY AND IMMUNOMODULATORY ACTIVITY OF AQUEOUS EXTRACT OF POLYHERBAL FORMULATION IN ALBINO WISTAR RATS

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ABSTRACT

Bioactive component and the toxic effect of plants material play a pivotal role in ascertaining the integrity of plant material. This study was aimed at determining the qualitative phytochemical screening, acute toxicity study and immunomodulatory activity of polyherbal formulation (PHF) in Albino Wistar Rats. Standard methods and procedures were carried out to determine the phytochemical constituents of the formulation. A total of 36 Wistar rats were used for the study. Lorke's methods were used for acute toxicity study. A total of 24 Wistar rats 4 per group were divided into 6 groups. Group 1 received normal saline, Group 2 Levamisole hydrochloride 50 mg throughout the intervention, Groups 3 received Cyclophosphamide 200 mg at the beginning of the study, Group 4-6 received varying doses of PHF daily for 14 days after which blood sample was collected, serum cytokines (IL-10 and IL-12) were measured using quantitative enzyme linked immunosorbent assay technique. The result of this study shows the presence of some constituents in the PHF with the absence of Anthroquinones. The LD50 of PHF was above 5000 mg/kg/bw and no mortality was reported. Serum level of IL-12 significantly increase in dose dependant manner across the treatment group ($p<0.001$),

whereas IL-10 is inconsistent. Therefore, it may be concluded that the PHF has rich source of phytoconstituent and practically safe with potential immunomodulatory activity on IL-12.

KEYWORDS: Cytokine, Immunomodulation, Lethal Dose $_{50}$, Plants medicinal, Phytochemicals.

INTRODUCTION

Medicinal plants are a very rich source of bioactive substances which are use in the treatment of different disease condition.[1] This underscore the crucial importance of Secondary metabolites as biosynthesized in plants for different purposes including growth regulation, inter and intra-specific interactions and defense against predators and infections.[2] Herb-herb combinations also known as polyherbal therapy have been used in Chinese medicinal practice for thousands of years, yet scientific evidence of their therapeutic benefits is lacking.[3] Drug combination often produces a promising effect in treatment of diseases over a single drug, the concept of drug combination has been well established in Western medicine and remarkable success has been achieved over a decade.[4]

A Polyherbal formulation called "*Garjin"* locally consists of five plant materials namely: *Acacia polyacentha wild* (Black cutch- *Bark)*, *Bauhinia rufescens lam* (orchid bush-Bark), *Acacia Senegal* (Gum Arabic tree-Bark), *Adensonia digitata* (Baobab-leaves), *Allium sativum* (Garlic-Yellow-bulb). *Acacia polyacentha wild* is popularly known for its astringent and antioxidant activities.[5] It exhibits various immunopharmacological effects like antipyretic, anti-diarrhoeal, hypoglycaemic, hepatoprotective, antioxidant activities, applied externally to ulcer, boils and skin eruptions etc.[6] *Bauhinia rufescens lam* is use for the treatment of diarrhea, dysentery, Jaundice, diabetes mellitus, ophthalmic diseases, syphilis and other venereal diseases and to reduce fever.[7] *Acacia senegal* is known to be beneficial in kidney diseases, act as an anti-oxidant,[8] and is known for its analgesic, astringent, emollient, liver tonic, antipyretic, anti-asthmatic, oral cavity lesions bleeding, bronchitis, gonorrhoea, leprosy, typhoid fever and upper respiratory tract infections properties.[9] *Adensonia digitata lam* is known native tree to Africa, baobab has numerous biological properties including antimicrobial, anti-malaria, diarrhoea, anaemia, asthma, antiviral, anti-oxidant and anti-inflammatory activities amongst others.[10] Other therapeutic importance includes the treatment of bronchial asthma, dermatitis, sickle cell anaemia, diuretic, anti-diabetic, diarrhoea, dysentery, laxative, hic-cough in children, anti-oxidant, anti-inflammatory, antidote for poison, anti-trypanosome uses.[11][12] *Allium sativum* is used widely in food and

medicine.[13] The aqueous garlic extract exerts antioxidant action by removing reactive oxygen species and enhancing cellular antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.[14] In addition, garlic represents an important source of antioxidants due to phytochemicals such as diallyl sulfide (DAS) and S-allyl mercaptocysteine.[15] Immunomodulation is a process by which an organism's immune system is modified and regulated by certain molecules called immunomodulators.[16] Immunomodulation includes the innate and adaptive arms of the immune system through the dynamic regulation of messenger molecules such as cytokines, facilitating phagocytic function, adhesion molecules, nitric oxide, hormones, neurotransmitters and others peptides among others.[17][18] The role of cytokines as immunomodulators is considered very important for the cure of the disease; they are autocrine, paracrine or endocrine in action and can exert a synergistic or antagonistic effect on their own production.[19] This PHF was_claimed to have immunomodulatory activities by a local herbal medical practioners in Kano, Nigeria. Consequently, it is used by some people in the state. Certainly, previous study shows it has potential immunomodulatory effect on Neutrophils function and humoral immune response.[20] However there is no scientific information on its phytochemical constituents or acute toxicity as well as cytokines. Therefore this study was set to provide this vital information.

MATERIALS AND METHODS

Plant materials

Plant material was collected from Al-Mustakshif Medical Health Centre, Kano, Nigeria (RC: 1393615). The plant materials were washed under running tap water to remove the surface pollutants and the whole plants were allowed to shade-dried and made into a coarse powder which was passed through a 40-mesh sieve to get a uniform particle size. A 60 g of the powder was dissolved into 1000 ml of distil water.The preparation was left to soak for 24 hours in a water bath set at 40^{o}C. The preparation was filtered using Whatman No. 1 filter paper. The resultant filtrate was concentrated to dryness at 40^{o}C under reduced pressure.[21]About 0.1 g of the dried extract was dissolved in 1 ml of distilled water and this served as stock.

Animals

A total of 36 Wistar rats of 16-18 weeks old, weight between 126-148g of either sex (13 Females; 13 Males) were used in this study. The animals were purchased from the

Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Ahmadu Bello University (ABU), Zaria, Nigeria 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.* Out of 36, 12 were used for acute toxicity study while 24 were used for cytokines analysis. The animals were maintained under the Institutional standard laboratory conditions for experimental animal and the principles of laboratory animal care guidelines were adopted.[22] The study was approved by Faculty of Veterinary Medicine Animal Research Ethics Committee (FAREC) with reference number as UDUS/FAREC/2019/AUP-RO-3.

Phytochemical Screening of Polyherbal Formulation

The phytochemical screening was carried out on the crude aqueous extract of the polyherbal formulation at Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmacological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The following phytochemicals were screened Alkaloids,[23] Steroids, Terpenoids, Vitamin and Saponins,[24] Cardiac Active Glycosides and Flavonoids,[25] Anthroquinones,[26] Tannins, Polyphenol, Carbohydrates and Proteins.[27]

Acute Toxicity Studies

This was carried out in the animal house of Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. The procedure was described by Lorke's.[28].

IMMUNOMODULATORY ACTIVITY ON CYTOKINES

Animal grouping and treatment

A total of 24 Wistar rats were randomly divided into six groups and each group contains fourrats. Group 1 received 10ml/kg b.w. of normal saline, Group 2 received 50mg/kg b.w. of Levamisole Hydrochloride (Guangehou Kafen Biotech Co., Ltd, China). Groups 3 received subcutaneous injection of 200 mg/kg b.w. of Cyclophosphamide (Guangehou Kafen Biotech Co., Ltd, China). At day one, Group 1 - 6 received 250, 500 and 1000 mg/kg bw of PHF respectively, for 14 days orally.

Blood collection and processing

On day 15**,** 2 ml of blood sample was collected by cardiac puncture from each Wistar rat under mild chloroform anaesthesia. The blood samples were transferred into clean plain tubes

allowed to clot within 30 minutes of collectionthen centrifuged at 500 g for 5 minutes to obtain neat serum and then transferred into a labelled cryovials then immediately stored at -20^{o}C until used.

Cytokines analysis

To measure the serum concentration of cytokines (i.e. IL-10 and IL-12), sandwich-ELISA technique (Sunlong Biotech Co, Ltd., China) was used. The procedure was performed according to manufacturer's instructions. The assay range for IL-10 is 1.6pg/ml-80 pg/ml and assay sensitivity is 0.5pg/ml. For IL-12, assay range is 3pg/ml-200 pg/ml and assay sensitivity is 0.5 pg/ml.

Statistical Analysis

The statistical analysis was carried out using SPSS 21 Software package (IBM, USA). Test for normality was performed to ascertain normal distribution of the variables. Data was not normally distributed based on tests of normality results: Shapiro-Wilk, supported by Q-Q plot. Therefore the results obtained are presented as Median. Kruskal Wallis test was carried out to explore differences on variables across the groups. The p value ≤ 0.05 was used to determine the level of statistical significance.

RESULT

Phytochemical constituents

The physical properties of polyherbal formulation extract shows gummy texture and brown colour and the percentage yield of the extract after aqueous extraction was 31.1% (Table 1). The Preliminary phytochemical screening of aqueous extract of the polyherbal formulation shows that it contains the following constituents Alkaloid, Terpenoids, Steroid, Cardiac Active Glycoside, Flavonoids, Saponins, Tannins, Polyphenols, Vitamins, Carbohydrate, Photosterols, Protein, however Anthroquinones is absent.

Acute toxicity

The Lethal Dose (LD_{50}) of the aqueous extract of polyherbal formulation was above 5000 mg/kg/bw. No any mortality was recorded after 24 hours of observation (Table 2).

Immunomodulatory activities on cytokines

The study revealed that there is a statistically significance difference in concentration of IL-10 across the six groups (χ^2 =26.81, p <0.001). Group 2 recorded a highest concentration

median score (Median =43.02pg/ml) and the least is group 3 (Median = 10.55 pg/ml). Similarly there is statistically significance difference in concentration of IL-12 across the six groups (χ^2 =32.32, p<0.001). Group 2 shows a highest concentration median score (Median =196.15 pg/ml) compared with the other groups and the least is group 3 (Median = 20.74 pg/ml) depicted from Table 3.

Table 1: Physical properties of polyherbal formulation extract and the percentage yield of the extract after aqueous extraction.

Plant material	Plant part	Colour	Type of extraction	% yield	Texture	Colour
Acacia polyacentha wild	Bark	Light-brown	Aqueous extract	31.2%	Gummy	Brown
Bauhinia rufescens lam	Bark	Dark-brown				
Acacia senegal	Bark	Light-brown				
Adensonia digitata	leaves	Green				
Allium sativum	Bulb	Light-yellow				

Table 2: Lethal Dose (LD_{50}) of the aqueous extract of PHF in Albino Wistar rats.

Dose (mg/kg/b.w.)	Observation	
	First phase	Second phase
10	0/3	-
100	0/3	-
100	0/3	-
1600	-	0/1
2900	-	0/1
5000	-	0/1

0/3 means none of the wistar rat died out of the three rats in the group; 0/1 means none of the wistar rat died in each group of one wistar rat after 24 hours of experiment.

Table 3: Effect of aqueous extracts of PHF on serum concentration of IL-10 and IL-12 in Wistar Albino rats.

Group (n=4)	Dose (kg/b.w.)	IL-10 (pg/ml)	IL-12 (pg/ml)
		Median	Median
I (NS)	10ml	34.14	54.14
II (LEV)	50 mg	43.02	196.15
III (CYP)	200mg	10.55	20.74
IV (PHF)	250mg	32.86	77.28
V (PHF)	500 mg	36.38	126.35
VI (PHF)	1000 mg	35.39	192.48
Kruskal Wallis Test		**χ^2= 26.81, p< 0.001**	**χ^2= 32.32, p<0.001**

NS= Normal saline; **CYP**= Cyclophosphamide; **LEV=** Levamisole hydrochloride

DISCUSSION

Polyherbal formulations are abundantly gaining popularity globally as compared to allopathic medicine for the treatment of different types of ailments,[29] the integrity of any plant material can be measured by its bioactive constituent and its safety to use.[30]

Our result on the Phytochemical screening shows that the polyherbal formulation contain the followings Alkaloid, Terpenoids, Steroid, Cardiac Active Glycoside, Flavonoids, Saponins, Tannins, Polyphenols, Vitamins, Carbohydrate, Photosterols, Protein, however, there is no Anthroquinones. This finding is in agreement with some previous studies which reported some individual plants with similar constituents with that of the formulation, *Acacia senegal*,[6][31] *Bauhinia rufescens Lam*[7] and *Allium sativa*.[9][32][33] However, another study reported the presence of Anthroquinones in *Adensonia digitata*.[34] The disparity may be confined to the uniqueness in the combination of the formulation and probably as a result of differences in the geographical location, weather condition and soil type of the plants. Plant of same species may have variation in their constituent due to differences in geographical location, weather condition, soil type etc.[35] Furthermore, herbs to herbs combination may lead to variation in constituent of a formulation due to the individual herbs proportion that makes the formulation.[36]

This study revealed that the LD50 of the formulation is greater than 5000 mg/kg/bw, therefore was thought to be safe.[28] Furthermore any compound with oral LD50 of 5000 mg/kg/bw or more should be considered as practically harmless.[37] This finding is in line with some previous study which reported that *Adensonia digitata* had LD50 of 8000 mg/kg,[38] *Adensonia digitata*[39] *Acacia senegal*[40] and *Acacia polyacentha* bark extract.[41] However on contrary some studies reported low LD50 for instance LD50 of *Allium sativum* is 650 mg/kg,[42] *Bauhinia rufescens* is 1265 mg/kg.[43] Of note some of these plants are individual constituents of the formulation. The disparity may be as a result of the plant material combination, difference in specie type of the plant, the animal species which may differ in toxicity effect and route of administration of the plant material.[44]

In this study we found that there is irregular increase in IL-10 concentration across the treatment groups. The increase is not consistent with dose increase of the PHF. This finding suggests that, there is inconsistent activity of the PHF on secretion of IL-10. It displays both immunosuppressive and immunostimulating activities.[45] The cytokine blocks the production of pro-inflammatory cytokines such as IFN-γ, IL-12 and Th2-derived cytokine like IL-5, but

induced IgE synthesis of IL-4.[46] As IL-10 in turn strongly inhibits antigen presenting cell-derived IL-12 production.[47] Some cytokines such as IL-12 antagonistically oppose the functions of IL-10.[48] Our findings is in conformity with the study of Jeong[49] which reported that aqueous extract of Jeo-Dang-Tang a polyherbal formulation down regulate or stabilized IL-10 production, however study by Luo[50] disagree with our findings, they reported that aqueous extract of Qing-huo-bai-du-yin a polyherbal formulation up regulate IL-10 production. Polysaccharides in herbs have been proven to possess potent immunostimulating effect by significantly decrease IL-10 expression in mice.[51]

This study finding revealed that after the treatment with PHF, there is significant increase in IL-12 concentration with corresponding increase in PHF dose across the treatment groups. These findings indicated that, PHF has potential effect on the secretion of IL-12. Cytokine production is mediated by monocytes, macrophages, dendritic cells and other antigen-presenting cells. Indeed, proliferation of both T and NK cells is enhanced in response to IL-12, and contributes to its robust immunomodulatory activity.[52] Important property of IL-12 is its ability to induce production of IFN-γ from immune effector cells. This propensity for promoting IFN-γ production is a means by which IL-12 facilitates cellular interactions that bridge innate and adaptive immune responses.[53] Our finding was consistent with the findings of Kurokawa[54] which reported that Kakkon-to a polyherbal formulation up regulate IL-12 production. Furthermore, Saponins stimulate lymphocytes and IL-12.[55]

CONCLUSIONS

The polyherbal formulationis rich in phytochemical that may be responsible for some of the pharmacological and immunomodulatory activitiesas claim by the herbalist. The formulation may be practically safe as no mortality observed in the study and the LD_{50} is above 5000 mg/kg/bw. The PHF demonstrated potential immunomodulatory activity on serum IL-12 level, however inconsistent on the concentration of IL-10.

Conflict of Interests

The authors declare that there is no conflict of interests.

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REFERENCES

1. Shukla S, Mehta A. Comparative phytochemical analysis and in vivo immunomodulatory activity of various extracts of *Stevia rebaudiana* leaves in experimental animal model. Front Life Sci, 2015; 8(1): 55–63.
2. Ukwuani AN, Abubakar MG, Hassan SW, Agaie BM. Toxicological Studies of Hydromethanolic Leaves Extract of *Grewiacrenata.*Int. J. Pharm. Sci. Drug Res, 2012; 4(4): 245-49.
3. Che CT, Wang ZJ, Chow MSS, Lam CWK. Herb-herb combination for therapeutic enhancement and advancement: Theory, practice and future perspectives. Molecules, 2013; 18(5): 5125-141.
4. Aslam MS, Ahmad MS, Mamat AS, Ahmad MZ, Salam F. An Update Review on Polyherbal Formulation: A Global Perspective. Sys Rev Pharm, 2016; 7(1): 35-41.
5. NdjateuFST, TsafackRBN, Nganou BK, Awouafack MD, Wabo HK, Tene M, Tane P, EloffJN. Antimicrobial and antioxidant activities of extracts and ten compounds from three Cameroonian medicinal plants: *Dissotisperkinsiae (Melastomaceae), Adenocarpusmannii (Fabaceae) and Barteriafistulosa (Passifloraceae).*S Afr J Bot, 2014; 91: 37-42.
6. Gupta A, Chaphalkar SR. Cytotoxic and Antiviral Activity of *Acacia Catechu* on Human Peripheral Blood Mononuclear Cells: Indonesian J. Pharm, 2016; 27(2): 111 – 16.
7. Koya MS, Elamin NA., Idid SZ. Isolation and Identification of Flavonoids from leaves of *Bauhinia rufescens Lam*;J Fundam Appl Sci, 2014; 30: 35-52.
8. Singh BR, Dubey S, Siddiqui MZ. Antimicrobial Activity of Natural Edible Gums World J Pharm Sci, 2015; 3(11): 2217-221.
9. Okoro SO, Kawo AH, Arzai AH. Phytochemical Screening, Antibacterial and Toxicological Activities of *Acacia Senegal* Extracts. BAJOPAS, 2012; 5(1): 163–70.
10. Rahu LJ, Jain MK, Singh SP, Kamal RK, Anuradha Naz A, Gupta AK, MrityunjaySK. *Adansonia digitata* L. (baobab): a review of traditional information and taxonomic description. Asian Pac J Trop Biomed, 2015; 5(1): 79-84.
11. Sundarambal M, Muthusamy P, Radha R, Jerad Suresh A. A review on *Adansonia digitata* Linn. J Pharmacogn Phytochem, 2015; 4(4): 12-6.

12. Sharma A, Rangari V.Immunomodulatory activity of methanol extract of Adansonia digitata L.Trop J Pharm Res, 2016; 15(9): 1923-927.
13. Sulaimana FA, Kazeem MO, Waheeda AM, Temowoa SO, Azeeza IO, Zubair FI, Adeyemi TA, Nyang A, Adeyemi OS. Antimicrobial and toxic potential of aqueous extracts of *Allium sativum, Hibiscus sabdariffa and Zingiberofficinale* in Wistar rats. J Taibah Univ Sci, 2014; 8(4): 315-22.
14. Arreola R, Quintero-Fabián S, López-Roa RI, Flores-Gutiérrez,EO, Juan Pablo Reyes-GrajedaPJ, Carrera-Quintanar L, Ortuño-Sahagún D. "Immunomodulation and Anti-Inflammatory Effects of Garlic Compounds," J Immunol Res, 2015; 2015 Article ID 401630: 13.
15. Badr GM, Al-Mulhim JA. The protective effect of aged garlic extract on nonsteroidal anti-inflammatory drug-induced gastric inflammations in male albino rats. Evid Based Complement Alternat Med, 2014; 2014: 759642.
16. Gomes A, Poulami D, Amrita S, Subir CD, Aparna G. Black tea (*Camellia sinensis*) extract as an immunomodulatory against immunocompetent and immunodeficient experimental rodents. Orien Pharm Exp Med, 2013; 14(1): 37–45.
17. Burns JJ, Lijun Z, Ethan WT, Kevin S. The influence of traditional herbal formulas on cytokine activity. J toxicol, 2009; 23(3): 1-20.
18. Nage PN, Rajeshwari P. An Overview on Immunomodulators. Int. J. Curr. Pharm.Clin. Res, 2014; 4(2): 108-14.
19. Dhama K, Saminathan M, Siju SJ, Mithilesh S, Karthik A, Ruchi T, Lakshmi TS, Yashpal SM, Raj KS. Review Article Effect of Immunomodulation and Immunomodulatory Agents on Health with some Bioactive Principles, Modes of Action and Potent Biomedical Applications. Int J pharmacol, 2015; 11(4): 253-90.
20. Isah SY, Mohammed HY, Hamid KM, Usman M, Mustapha UK. Effect of Polyherbal Formulation on Neutrophil function and TDAR in Wistar albino rat. ejpmr, 2018; 5(12), 563-68.
21. Gadanya AM, Muhammad SU. Hypolipidemic effect of oral administration of aqueous leaf extract of *Senna occidentalis* in rats. Niger J Basic Clin Sci, 2018; 15(1): 68-72.
22. Wolff AV, Smith PD. Compliance at the Institutional and Programmatic Level. Lab Animal. 1994; 23(8): 28-29.
23. Trease GE, Evans WC. A Textbook of Pharmacognosy. (13th ed.). London: Bailliere Tinall Ltd; 1989. 71-78 p.

24. Sofowora A. Medicinal plants and traditional medicine in Africa. (2nd ed.). Ibadan, Nigeria: Spectrum Books Limited; 1993. 289 p.
25. Brain KR, Tuner TD. The practical evaluation of phytopharmaceuticals. Bristol: Wright-Scientechnica, 1975. 57-58 p.
26. Culie A. Methodology for the analysis of vegetables and drugs. NIDO Romania: Chemical Industry Division; 1997. 24-7 p.
27. Harborne AJ. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. (3rd ed.). Netherlands: Springer; 1998. 34-36 p.
28. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol. 1983; 54(4): 275-87.
29. Isah SY, Ahmad I M, Hamid KM, Abdulhadi AU, Abdullahi HL, Danladi SB, Ahmad MB, Rogo LD. Effect of Aqueous Leaves Extract of *Balanite Aegyptiaca on* Some Biochemical and Haematological Parameters in Wistar Rats. Sok J MLS, 2017; 2(3): 134–41.
30. Chouhan S, Sharma K, Guleria S. Antimicrobial Activity of Some Essential Oils-Present Status and Future Perspectives. Medicines (Basel), 2017; 4(3): E 58.
31. Sharma R, Kaur S. Antimicrobial and phytochemical Screening of *Trikuta*-traditional foog of western Rajasthan. Indian J Tradit Know, 2017; 16(2): 270-276.
32. Garba I, Umar AI, Abdulrahman AB, Tijjani MB, Aliyu MS, Zango UU, Muhammad A. Phytochemical and Antibacterial Properties of Garlic Extracts. BAJOPAS, 2013; 6(2): 45-48.
33. Huzaifa U, Labaran I, Bello AB, Olatunde A. Phytochemical Screening of Aqueous Extract of Garlic (*Allium sativum)* bulbs. Rep Opinion, 2014; 6(8): 1-4.
34. Abiona DL, Adedapo Z, Suleiman MK. Proximate Analysis, Phytochemical Screening and Antimicrobial Activity of Baobab (*Adansonia digitata)* Leaves. IOSR-JAC, 2015; 8(5): 60-65.
35. Mäkinen H, Kaseva J, Virkajärvi P, Kahiluoto H. Shifts in soil–climate combination deserve attention. Agric For Meteorol, 2017; 236-246.
36. Kunle OF, Egharevba HO, Ahmadu PO. Standardization of herbal medicines - A review.: Int. J. Biodivers. Conserv, 2012; 4(3): 101-112.
37. Hodge A, Sterner B. Toxicity classes. In: Canadian center for occupational Health and safety. [Online]. 2005 [cited 2018 Nov 28]. Available from: http://www.ccohs.ca/oshanswers/chemicals/id50.htm.

38. Elsaid FG. The Effect of Seeds and Fruit Pulp of *Adansonia digitata L*. (Baobab) on Ehrlich Ascites Carcinoma. Food Nutr Sci., 2013; 4(8): 38-46.
39. Lawal B, Shittu OK, Oibiokpa FI, Mohammed H, Umar SI, Haruna GM. Antimicrobial evaluation, acute and sub-acute toxicity studies of *Allium sativum*. J Acute Dis., 2016; 5(4): 296–301.
40. Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Frutos MJ, Galtier P, Gott D, Gundert-Remy U, Lambre C, Leblanc J-C, Lindtner O, Moldeus P, Mosesso P, Oskarsson A, Parent-Massin D, StankovicI, Waalkens-Berendsen I, Woutersen RA, Wright M, Younes M, Brimer L, Christodoulidou A, Lodi F, Tard A, Dusemund B. EFSA ANS Panel (EFSA Panel on Food Additives and Nutrient Sources added toFood), Scientific Opinion on the re-evaluation of acacia gum (E 414) as a food additive. EFSA Journal, 2017; 15(4): 4741.
41. Okpanachi AO, Adelaiye AB, Dikko AAU, Kabiru M, Mohammed A, Tanko Y. Evaluation of the Effect of Aqueous-methanolic Stem Bark Extract of *Acacia polyacantha*on Blood Glucose Levels of Alloxan Induced Diabetic Wistar Rats. Int. J. Anim. Veter. Adv, 2010; 2(3): 59-62.
42. Ekong MB, Muonagolu NJ, Peter AI, EkandemGJ, Ekanem TB. Allium sativum extract affects medial prefrontal cortical cytoarchitecture. Histol. Cytol. Embryol, 2017; 1(4): 1-6.
43. Hassan H, Sule M, Musa A, Oyinloye F. Phytochemical and anti-diarrheal studies of *Bauhinia rufescens* Lam. J Pharm Bioreso, 2008; 5(2): 54-62.
44. Ko KS, Koh DC, Kong IC. Evaluation of the Effects of Nanoparticle Mixtures on Brassica Seed Germination and Bacterial Bioluminescence Activity Based on the Theory of Probability. Nanomaterials (Basel), 2017; 7(10): E344.
45. Mannino MH, Zhu Z, Xiao H, Bai Q, Wakefield MR, Fang Y. The paradoxical role of IL-10 in immunity and cancer. Cancer Lett., 2015; 367(15): 103–107.
46. Abbas AK, Murphy KM, Sher A. Functional diversity of helper T Lymphocytes. Nature, 1996; 383: 787-793.
47. Ma X, YanY, Zheng H, Du Q, Zhang L, Ban Y, Li N, Wei F. Regulation of IL-10 and IL-12 production and function in macrophages and dendritic cells. F1000 Res., 2015; 4: 1-13.
48. Xiaojing M, Wenjun Y, Hua Z, Qinglin D, Lixing Z, Yi B, Li N, Wei F. Review Regulation of IL-10 and IL-12 production and function in macrophages and dendritic cells. F1000 Res, 2016; 4: 1-13.

49. Jeong HJ, Chung HS, An HJ, Kim JB, Lee EM, Park EJ, Jang CH, Hong SH, Kim HM. Immune-enhancement effect of the herbal combination Allergina. Clin. Chim. Acta, 2003; 337: 77–84.
50. Luo CQ, Zhou JD, Chen DJ. Effect of Chinese traditional medicine mixture on inflammatory response in rats with severe burn. Yi Xue Ban, 2004; 29: 32–4.
51. Li J, Li QW, Han ZS, Lu WZ. Antitumor and Immunomodulating effect of polysaccharides isolated from *solanum nigrum* L. Phytother Res., 2009; 23: 1524-30.
52. Marçais A, Viel S, Grau M, Henry T, Marvel J, Walzer T. Regulation of Mouse NK Cell Development and Function by Cytokines. Frontiers Immunol*oy*, 2013; 4: 450-58.
53. Watford WT, Moriguchi M, Morinobu A, John JS. The biology of IL-12: coordinating innate and adaptive immune responses. Cytokine & Growth Factor Rev., 2003; 14: 361–368.
54. Kurokawa M, Tsurita M, Brown J, Fukuda Y, Shiraki K. Effect of interleukin-12 level augmented by Kakkon-to, a herbal medicine, on the early stage of influenza infection in mice. Antivir Res., 2002; 56: 183–88.
55. Cho JY, Kim AR, Yoo ES, Baik KY, park MH.Ginsenosides from panax ginseng differently regulate lymphocyte proliferation. Planta Med., 2002; 68: 497-500.