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IN VITRO IMMUNE-MODULATORY POTENTIAL OF CRUDE EXTRACT OF LEAF OF *ALBIZIA GUMMIFERA* AGAINST STIMULATED PERIPHERAL BLOOD MONONUCLEAR AND RAW CELLS

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Abstract

Background: *Albizia* species including *A. gummifera* have been used in folk medicine for the treatment of various conditions by many cultural groups. Apart from its anti-parasitic and antimicrobial activity associated with its use, *Albizia gummifera* have not been investigated for either its anti-inflammatory properties or anti-proliferative effect *in vitro*.

Materials and Methods: Extracted acetone crude extract of plant material was tested for the presence of various secondary metabolites using chemical tests while the anti-proliferative effect against peripheral blood mononuclear were tested using the WST-1 assay. Nitric oxide production by RAW cell line exposed to extract was also tested using Griess assay as well as the production of interleukin- 2 in medium of cultured PMBC using an enzyme-linked-immunosorbent assay (ELISA) kit.

Results: Phytochemical testing of acetone leaf extracted was shown to be positive for flavonoids, saponins and tannins. The extract was shown to dose-dependently inhibited proliferation of mononuclear cells while promoting that of RAW cells ($p<0.05$). The production of IL-2 by mononuclear cells and nitric oxide release by RAW cells were inhibited and stimulated respectively ($p<0.05$) in both cell types. The extract was also shown to elicit significant anti- and pro-inflammatory potential at concentration above 20 and 40 µg/ml, in both cell types respectively.

Conclusion: Further laboratory research is required to elucidate the anti- and pro-inflammatory biochemical pathways as shown by the *in vitro* immune-modulatory modalities of this plant species. It is also important to further identify bioactive entities of *Albizia gummifera* responsible for the observed activities.

Key words: *Albizia gummifera*, cell proliferation, Interleukin-2, nitric oxide, phytochemistry.

Introduction

Albizia gummifera is a native African herb located in Angola, Cameroon, Democratic Republic of Congo, Ethiopia, Kenya, Madagascar, Nigeria, Tanzania, Uganda, Zambia and Brazil - where it does not naturally grow. *Albizia gummifera* inhabits forests (low- or upland rain-forest) as well as open locations near forests. It is a 4.5 –30 m large deciduous tree, with branches apexing to a flat top. The plant is known by different names in Swahili such as mshai, mkenge and mchai mbao, and peacock flower in English. The name gummifera refers to the gum that is produced by the plant when the bark is cut (Orwa et al., 2009).

The various portions of *A. gummifera* exhibit various medicinal properties such as anti-parasitic (malaria and trypanosomiasis), antibacterial (*Bacillus subtilis, Staphylococcus aureus*, *Escherichia coli, Neisseria gonorhea*, *Pseudomonas aeruginosa*) activities and for the relief of stomach aches (Kokwano, 1976; Orulla, 1996; Rukunga et al., 2007; Tefera et al., 2010).

The anti-bacterial and immune-modulatory potential of various medicinal plants in Limpopo Province, South Africa has been reported for activity on leukocyte function in numerous cell lines *in vitro* by co-workers in the Department of Biochemistry, Microbiology and Biotechnology (BMBT), University of Limpopo (Masoko et al., 2010). New species used by traditional herbalists for treating various ailments in our province have been acquired by colleagues in the Department of BMBT (Mokgotho, et al., 2013). Out of the six plant species screened, we

here report on the inflammatory potential of *A. gummifera* which was shown to exhibit activity in our experimental models of immune-pharmacology.

There is presently an increase in research activities aimed at identifying anti-inflammatory agents. Our efforts and focus for pursuing this research activity are to identify and show proof of activity as well as expanding the ethno-botanical library of anti-inflammatory medicinal agents.

Materials and Methods

Chemicals and Reagents

Unless otherwise indicated, all chemicals and reagents were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA).

Plant leaf and extraction procedure

Leaves of *A. gummifera* were collected from White River, Mpumalanga Province, South Africa in March 2009. The accession number UNIN 11 937 of the specimen was deposited in the University of Limpopo Herbarium. The leaf material was air-dried and extracted using acetone according to the Soxhlet extraction method (Ehrman, 1994). The plant powder (20 g) was extracted in 200 ml of acetone (w/v).

Phytochemical analysis using chemical methods

Phytochemical analysis was performed on the extract to identify chemical constituents using standard methods (Singleton et al., 1999).

Test for tannins

Water (5ml) was added to 0.5g of plant extract and heated in a water bath. The sample was cooled and to it, few drops of 0.5% iron III chloride ($FeCl_3$) was added. The appearance of green or dark blue precipitate indicated a positive reaction for the presence of tannins.

Test for alkaloids

Sample (2g) was heated in a test tube containing 25ml of 1% HCl for 15 minutes in boiling water bath. The suspension was then filtered and 5 drops of Meyer's reagent (potassium tetraiodomecurate) was added to the filtrate. The appearance of a precipitate indicated a positive reaction for the presence of alkaloids.

Test for saponins

Plants leaf extracts (0.5g) was suspended in 5ml of water and heated for 5 minutes. The sample was cooled at room temperature and agitated for 10s. The absence of persistent foam indicated a positive reaction for the absence of saponins.

Test for triterpene and steroids

Sample (0.5g) was dissolved in 3ml of chloroform and few drops of acetic anhydride and concentrated H_2SO_4 was added. The absence of two layers upon the addition of H_2SO_4 indicated a positive reaction for the absence of both triterpene and steroids.

RAW 264.7 macrophage cell culture

Cells were obtained from Highveld Biologicals (Johannesburg, South Africa).These cells were grown in RPMI 1640 supplemented with 10% FCS, penicillin (100U/ml) and streptomycin in a humidified atmosphere of 5% CO_2 at 37°C.

Preparation of PBMC from human peripheral blood

PBMC were obtained by collecting venous blood (8ml) into specialised BD Vacutainer® CPT™ tubes according to the manufacturer's instructions (Beckton Dickinson, Johannesburg, South Africa).

Determination of cellular proliferative effects of leaf extract

After incubating the RAW 264.7 and PBMC cells (2 x 10^6 cells/ml) for 72 hours with or without leaf extract (5-160µg/ml) and stimulating with PMA (25ng/ml) and fMLP (1µM), proliferative activity was determined employing the WST-1 assay using a commercial kit according to the manufacturer's instructions (Roche Diagnostics, Johannesburg, South Africa).

Determination of nitric oxide production by RAW cell line

Cells were incubated for 24 hours after treatment and nitric oxide measured as its end-product, nitrite, in the supernatant using the Griess assay (Bogdan, 1998). Briefly, an aliquot of 100µl of culture supernatant of RAW cells was mixed with 100µl of Griess reagent (one part of 0.1% n-(1-naphthyl) ethylenediamine dihydrochloride in water and one part sulphanilamide in 5% H_3PO_4).The mixture was incubated for 10 min at room temperature in the dark and absorbance measured at 540 nm using a microplate reader (Beckman Coulter, Johannesburg, South Africa).

Determination of IL-2 production by PBMC

After treatment of cells with crude extracts for 24 hours, IL- 2 production in the culture medium was measured using an enzyme-linked-immunosorbent assay (ELISA) kit (Whitehead Scientific, Johannesburg, South Africa) according to the manufacturer's instructions.

Statistical analysis

Experimental data (repeats of 3-4 experiments performed in triplicates) was processed and analysed with GraphPad Prism Software Version 10.00 and data expressed as mean ± SEM. Statistical analysis was performed by the Student 's *t* test with $p<0.05$ considered significant.

Results

Phytochemical tests showed that the acetone extracts tested positive for flavonoids, saponins and tannins. The results of the proliferative activities of *A. gummifera* leaf extract on PBMCs and RAW cells are shown in Figures 1 and 2 respectively. The effects of the extract on IL-2 production by PBMCs and nitric oxide production by RAW cells are shown in Figures 3 and 4 respectively.

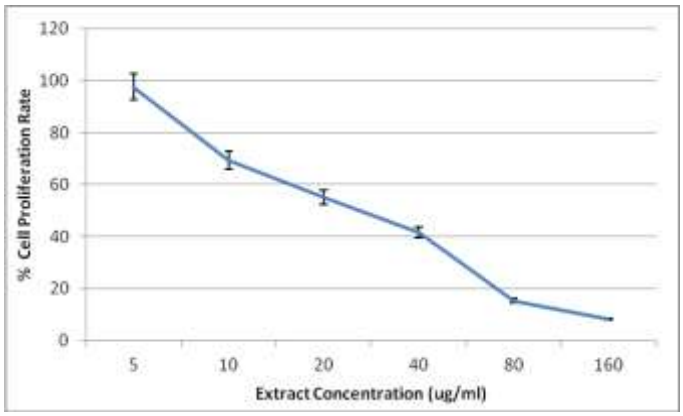

Figure 1: Inhibition of cell proliferation by leaf extract of *A. gummifera* on PBMCs *in vitro*. Results are expressed as the mean percentage inhibition of cell proliferation by the extract (5 – 160µg/ml) plus SEM of four different experiments conducted in tripliccates.

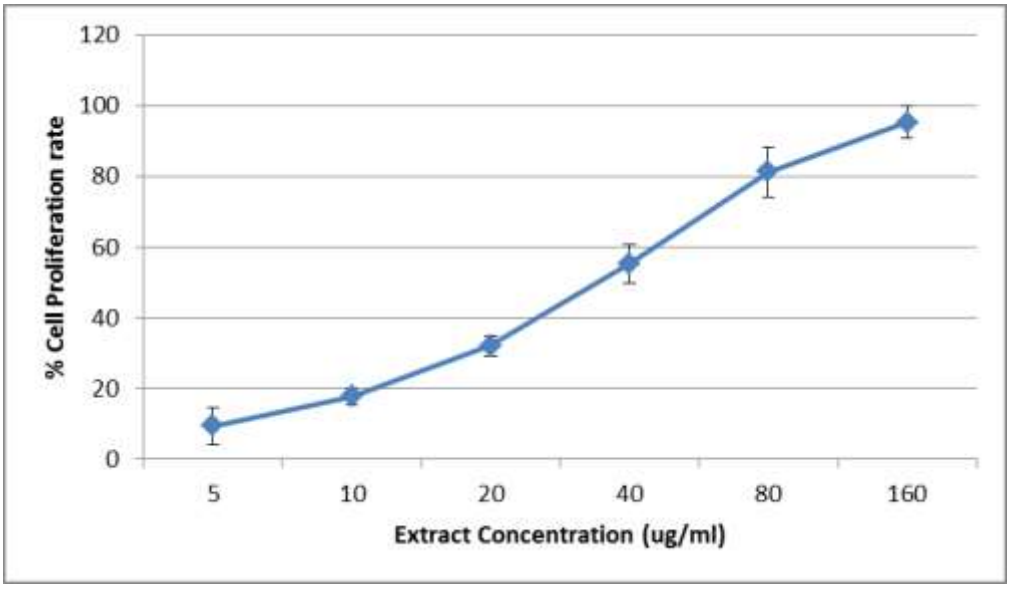

Figure 2: Stimulation of cell growth by leaf extract effect of *A. gummifera* of RAW cells *in vitro*. Results are expressed as the mean percentage promotion of cell proliferation by the extract (5 – 160µg/ml) plus SEM of four different experiments conducted in triplicates.

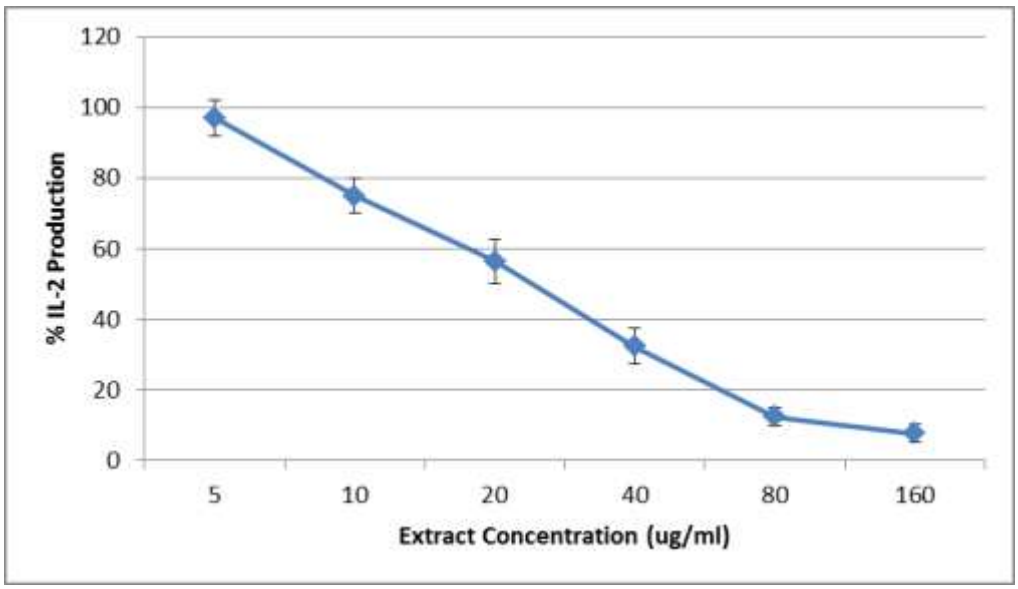

Figure 3: Effects of leaf extract of *A. gummifera* on the production of IL-2 by PBMCs *in vitro*. Results are expressed as the mean percentage of IL-2 production by the extract (5 – 160µg/ml) plus SEM of four different experiments conducted in triplicates.

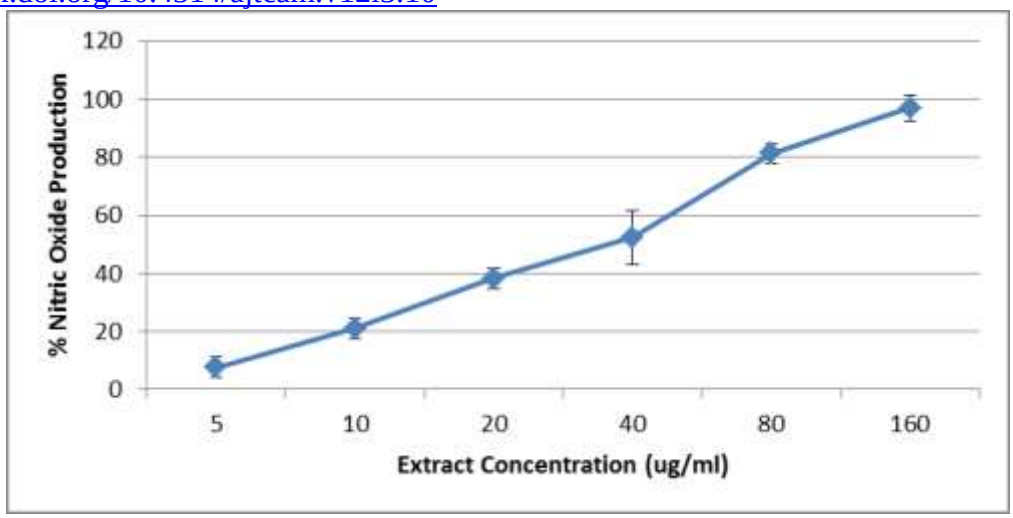

Figure 4: Effects of leaf extract of *A. gummifera* on the production of nitric oxide by RAW cells *in vitro*. Result is expressed as the mean percentage of nitric oxide production by the extract (5 – 160µg/ml) plus SEM of four different experiments conducted in triplicates.

Discussion and Conclusion

The RAW and PBMC cells were incubated with crude plant extracts of *A. gummifera* ranging from 5µg/ml to 160µg/ml for 72 hours. The extent of inhibition or promotion of cell growth was assessed using WST-1 assay. The results, as shown in Figure 1 and 2, indicates a dose-dependent cytotoxic effect on PBMC cells and growth stimulating effect on RAW cells respectively. The observed effects were shown to be evident and significant at concentrations of 40µg/ml for PBMC cells and 20µg/ml for RAW cells and higher ($p < 0.05$). The observed effects may partly be related to the ability of the extract to inhibit or promote the leukocyte lipid biosynthetic lipo- and cyclooxygenase pathways, since these metabolic pathways have been implicated in the release of inflammatory mediators such as histamine, prostaglandins and leukotrienes (Serhan, 2007)**.**

The plant extract was also shown to significantly inhibit IL-2 production by PBMCs at a concentration of 20µg/ml ($p < 0.05$), as represented in Figure 3. Interleukin-2 has been shown to be one of the markers of T cell activation (Boyman and Sprent, 2012), the presence of which confirms the anti-proliferative activity of the leaf extract of *A. gummifera* in this study. The extract was also shown to significantly ($p < 0.05$) promote the production of nitric oxide by RAW cells as shown in Figure 4. RAW cells are mouse derived macrophage cell line and the production of nitric oxide by phagocytes is used as a weapon against microbial pathogens (Marcinkiewics, 1997)**.** The result is also concomitant with the growth-promoting activity of the extract shown in Figure 2.

The current findings are preliminary and further extensive research activities will be conducted with the aim of isolating and screen of chemical entities isolated from the extracts for immuno-pharmacological profiling as well as identifying the biochemical pathways involved in the pro- and anti-inflammatory activities of the extract.

Acknowledgements

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Declaration of Conflict of Interest

No interest to declare.

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