Research Article

In-Vitro Evaluation of the Anti-Inflammatory Potential of Selected Jamaican Plant Extracts using the Bovine Serum Albumin Protein Denaturation Assay.

Yvonne A. Bailey-Shaw*[1], Lawrence A. D. Williams[1,2], Cheryl E. Green[1], Shawntae Rodney[1], Ann Marie Smith[1]
[1] Scientific Research Council, Product Research and Development Division, Hope Gardens Complex, Kingston 6, Jamaica West Indies.
[2] University of Technology, College of Health Sciences, 237 Old Hope Road, Kingston 6, Jamaica West Indies.
***Corresponding author's E-mail:** yvonneb@src-jamaica.org.

Received: 18-10-2017; **Revised:** 05-11-2017; **Accepted:** 18-11-2017.

ABSTRACT

Jamaica has a rich biodiversity, with high plant endemism. Indications are that a significant number of local plants are used in the treatment of various illnesses, including inflammatory conditions. Denaturation of proteins is one of the well documented causes of inflammation. This study seeks to evaluate the *in vitro* anti-inflammatory potential of the ethanolic leaf extracts of selected Jamaican plants. Ninety-nine (99) Jamaican plants were screened. Ethanolic extracts of their leaves were prepared by maceration. These were evaluated *in vitro* at concentrations of 0.25 µg/mL, 0.50 µg/mL and 1.00 µg/mL using the Bovine Serum Albumin (BSA) protein denaturation assay. Five of the most active crude extracts identified, were then column fractionated using solvent mixtures of ethyl acetate in hexane of increasing polarities. The collected fractions were pooled, based on their thin layer chromatography (TLC) profiles and further assayed, in order to determine the most active fractions. Aspirin was used as the reference anti-inflammatory drug. Crude extracts of *Cajanus cajan*, *Cinnamomum zeylanicum*, *Cordia alba*, *Mangifera indica* and *Tecoma stans*, significantly inhibited the denaturation of BSA exhibiting anti-denaturation activity of 62.31%, 49.61%, 65.47%, 72.60% and 61.50% respectively, in comparison to aspirin – (35.3%). Column fractionation of these crude extracts, resulted in increased inhibition of denaturation of BSA in fractions 5 (0.25 µg/mL), 16 (0.25 µg/mL, 0.50 µg/mL) and 12 (0.50 µg/mL) of *C. zeylanicum*, *T. stans* and *M. indica* respectively. However, complete loss of activity was observed in fractions of *C. cajan* and *C. alba*. The ethanolic extracts of *C. cajan*, *C. Zeylanicum*, *C. alba*, *M. indica* and *T. stans* possess significant anti-inflammatory activity. However, further investigations of fractions of *C. zeylanicum*, *T. stans* and *M. indica*, will be necessary for isolation of potential lead anti-inflammatory compounds. Crude ethanolic leaf extracts of *C. cajan* and *C. alba*, may be more efficacious than isolated compounds.

Keywords: Anti-inflammatory potential, BSA assay, *Cajanus cajan*, *Cinnamomum zeylanicum*, *Cordia alba*, *Mangifera indica*, *Tecoma stans.*

INTRODUCTION

Protein denaturation has been identified as the cause of inflammation. Indications are that when living tissues are injured, inflammation results. This is characterized by redness, pain, heat, swelling, as well as loss of function in the affected area. Disruption of the electrostatic, hydrogen, hydrophobic and disulphide bonds in the protein structure occurs. In addition, a complex array of enzyme activation, mediator release, cell migration, tissue breakdown and repair, occur, causing the protein to lose its molecular conformation and functions or become denatured. [1-4] It is therefore deduced that, compounds which are able to prevent these changes and inhibit thermally or heat induced protein denaturation, have potential therapeutic value as anti-inflammatory agents. [3]

Steroidal and non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used for the management of inflammatory conditions such as rheumatoid arthritis and other infectious diseases. They reportedly bind to plasma albumin, preventing or inhibiting the thermal denaturation of albumin. However, they often have toxic or secondary adverse effects resulting from prolonged use which cause damage to the liver, gastrointestinal tract as well as cardiovascular and renal failure. [1, 5-7] The need therefore exists, to explore alternative sources of anti-inflammatory drugs from plant origins. The perceived efficacy, low incidence of serious side effects or relative safety, compared to the synthetic alternatives, as well as the affordability of plant-derived drugs make this search worthwhile. In addition, the ethno-pharmacological uses of many medicinal plants extensively as crude extracts or as pure compounds, have generated considerable interest as it relates to the treatment of various medical conditions including chronic inflammatory diseases. With more than 80 % of the world's population currently relying on plant-derived medicines for their primary healthcare needs, screening of these plants for potential anti-inflammatory compounds could be a step toward the discovery of safer and more effective compounds. [6, 8-10]

Williams *et al.*,[11] proposed the stabilization of heat treated BSA by NSAIDs as an assay for replacing animals in the early stages of screening for non-steroidal anti-inflammatory drugs. Several research groups have since used the assay for validating compounds that could be of pharmaceutical interest. [2, 12] The folk medicinal literature of Jamaica indicates that several plants are used in the treatment of inflammation. [13-14] However, the scientific evidence is lacking. Ninety-nine Jamaican medicinal plants were therefore selected and the ethanolic leaf extracts screened for their stabilization effects on BSA using the *in vitro* BSA protein denaturation method. [11] Subsequently,

the five most active extracts were chosen and further separated by column chromatography for the possible isolation of lead anti-inflammatory compounds.

MATERIALS AND METHODS

Preparation of Crude Leaf Extracts

Leaves of ninety-nine (99) plants were collected from various locations across the island. They were taxonomically identified at the Department of Life Sciences' Herbarium (University of the West Indies, Mona, Jamaica). Samples were air dried under laboratory conditions (25 – 27 ^{o}C and 70 – 80 % Relative Humidity) for five days and then milled into a coarse powder. Ten grams (10 g) of dried powder of each plant was extracted with 95 % ethanol (200 mL) for five days. The resulting extracts were filtered and concentrated to oily residues *in vacuo* using a rotary evaporator (BÜCHI Rotovapor R-200) and then stored at 4 ^{o}C until required.

Bovine Serum Albumin Assay (BSA)

The anti-inflammatory activities of the crude and fractionated plant extracts were determined using a modified version of the BSA assay reported by Williams *et al.*[11] BSA solution (0.4%, w/v) was prepared in Tris Buffered Saline (one tablet is dissolved in 15 mL of deionized water to yield 0.05M Tris and 0.15M sodium chloride, pH 7.6 at 25 ^{o} C). The pH was adjusted to 6.4 with glacial acetic acid. Stock solutions of each plant extract were prepared in methanol at a concentration of 50 µg/mL or 0.005%, w/v. Respective aliquots of 5.0 µL, 10 µL and 20 µL representing concentrations of 0.25 µg/mL, 0.50 µg/mL and 1.00 µg/mL of the stock solutions were added to test tubes containing 1 mL of 0.4%, w/v BSA buffer solution. Both negative (methanol) and positive (aspirin) controls were assayed in a similar manner. The solutions were then heated in a water bath at 72 °C for 10 minutes, and cooled for 20 minutes under laboratory conditions. The turbidity of the solutions (level of protein precipitation) was measured at 660 nm in a Hach Spectrophotometer using an air blank. The experiments were conducted in duplicate and the mean absorbance values were recorded. The percentage inhibition of precipitation (protein denaturation) was determined on a percentage basis, relative to the negative control using the following equation:

$$*\%\ \text{Anti-Denaturation Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

* % Anti-Denaturation Activity = % Inhibition of Protein Denaturation = % Anti-inflammatory Activity

Gravity Column Fractionation of Crude Ethanolic Leaf Extracts

The five crude ethanolic leaf extracts; *Cajanus cajan* (Gungo), *Cinnamomum zeylanicum* (Cinnamon), *Cordia alba* (Duppy cherry), *Mangifera indica* (Julie mango) and *Tecoma stans* (Jamaican lilac) with anti-denaturation activity greater than 49 % were separated on a gravity column. A glass column with an internal diameter of 2.0 cm and length of 50 cm was wet-packed to 37 cm long with flash silica (230 – 400 mesh) using 1% ethyl acetate in hexane (starting solvent). One gram (1 g) of the crude ethanolic extracts was each dissolved in 10 mL of the starting solvent and then loaded on to the column using a Pasteur pipette. The column was eluted with 200 mL portions of solvent mixtures of increasing polarities; 1.0 % ethyl acetate - hexane, 5% ethyl acetate-hexane, 10 % ethyl acetate-hexane, 20 % ethyl acetate –hexane, 40 % ethyl acetate-hexane, 60% ethyl acetate – hexane, 80% ethyl acetate – hexane, 100% ethyl acetate and 50% ethyl acetate –methanol. Seventy (70) fractions of 20 mL portions were collected from each crude ethanolic extract. The profile of each fraction was then developed using thin layer chromatography (TLC) and thereafter pooled based on similarity in band formation. A total of 15-16 sub fractions were obtained for each crude plant extract on pooling. Each pooled fraction was concentrated to dryness and evaluated for anti-denaturation activity using the BSA assay described herein.

RESULTS

Of the ninety-nine (99) plants screened for anti-denaturation activity using the BSA protein denaturation assay, forty- two (42) exhibited 20% or greater inhibition of the denaturation of protein. Of these, six (6) extracts displayed greater than 49 % inhibition of protein denaturation (Table 1). Notably, although 20% inhibition of protein denaturation represents the minimum limit for potential anti-inflammatory agents, [15] plants which showed a minimum limit greater than 49% anti-denaturation activity were selected for further investigation, in order to increase the possibility of identifying potential lead anti-inflammatory compounds. These were the extracts of *C. cajan* (Gungo) 62.31 %, *C. zeylanicum* (Cinnamon) 49.61 %, Musa *sapientuum* (Banana –Lacatan variety) 51.94%, *C. alba* (Duppy cherry) 65.47 %, M. *indica* (Julie mango) 72.60 % and *T. stans* (Jamaican lilac) 61.50 %. *Musa sapientuum* was investigated in a previous study [16] and consequently was excluded from this investigation. Results for the remaining five plants are summarized in Table 2.

Table 1: Anti-denaturation activity of crude ethanolic leaf extracts (0.005% w/v) from ninety-nine (99) Jamaican plants.

Plant Scientific Name	Plant Common Name	% Anti-Denaturation Activity of Plant Ethanolic Extracts at Three Concentrations		
		0.25 µg/mL	0.50 µg/mL	1.00 µg/mL
Abultilon Trisulcatum	None Known	11.62	27.81	-7.03
Allium sativum	Garlic	28.51	27.56	32.38
Aloe vera	Sinkle Bible	33.02	-11.69	-50.00
Alysicarpus vaginalis	Medina	34.88	12.94	17.69
Annona reticulate	Custard Apple	9.73	1.49	-15.17
Annona squamosal	Sweet Sop	-29.46	-17.91	-2.88
Antigonon leptopus	Coralita	-5.04	-21.05	14.82
Artocarpus altilis	Breadfruit	5.75	1.01	-16.93
Asclepias curassavica	Redhead	-24.43	27.77	13.50
Azadirachta indica	Neem	-10.58	-12.07	-1.46
Bidens pilosa	Spanish Needle	-8.86	1.92	-3.19
Blighia sapida	Ackee	-45.81	-1.29	28.56
Bocconia frutescens	Celandine	9.54	-4.70	5.79
Boehmeria jamaicensis	Doctor Johnson	47.51	-18.89	-54.87
Bontia daphnoides	Kidney Bush	-56.25	18.25	-5.67
Bougainvillea spp.	Bougainvillea	-63.15	-25.32	15.31
Bryophyllum pinnatum	Leaf-of-Life	-31.51	6.60	-6.68
Cajanus cajan	Gungo	54.46	62.06	62.31
Caladium bicolor	Caladium	23.29	8.58	-30.23
Calotropis procera	French Cotton	-10.05	6.12	5.51
Capsicum annuum	Scotch Bonnett	-42.99	-7.82	-1.18
Cassia alata	King of the Forest	3.68	35.31	-8.02
Catharanthus roseus	Periwinkle	3.73	-22.18	-21.32
Cecropia peltata	Trumpet	-7.81	5.64	20.19
Chrysophyllum cainito	Star Apple	-96.94	-4.00	-41.49
Cinnamomum zeylanicum	Cinnamon	24.18	34.63	49.61
Cleome rutidosperma	Consumption Weed	42.16	-12.87	-40.43
Cleome viscosa	Wild Caia	10.90	30.96	32.68
Coccoloba uvifera	Seaside Grape	-31.86	-8.92	-36.77
Cocos nucifera	Coconut	-9.62	-4.31	4.38
Codiaeum varigatum	Garden Croton	22.57	22.02	-20.80
Cordia alba	Duppy Cherry (Sible Cherry)	65.47	39.53	-86.35
Crescentia cujete	Calabash Tree	7.33	17.46	8.50
Croton linearis	Wild Rosemary	29.23	-11.14	-44.23
Cucurbita spp	Pumpkin	17.58	25.00	-3.87
Curcuma longa	Turmeric	-20.86	-20.53	7.79
Cuscuta americana	Love Bush	21.24	12.42	-30.65
Cycloptis semicordata	Tall Fern	35.83	16.75	-16.34
Cymbopogon citratus	Fever Grass	-12.63	1.14	-30.00
Delonix regia	Poinciana	-40.32	-12.32	-53.73
Dieffenbachia spp.	Dieffenbachia (Ornamental)	-60.22	-32.91	-17.31
Dioscorea polygonoides	Wild Yam	-0.44	18.79	-25.81

Ervatamia divaricata	Coffee Rose	-62.82	-51.66	-3.02
Erythrina corallodendrum	Spanish Machette	18.37	-65.00	-67.02
Euphorbia hirta	Milk Weed	9.49	29.53	28.12
Fagara flava	Jamaican Satinwood	-16.16	-5.07	4.22
Ficus spp.	Evergreen	-15.85	15.06	-4.14
Gliricida sepium	Aaron's Rod (Quick Stick)	-29.73	7.94	-4.95
Guaiacum officinale	Lignum Vitae	-8.25	9.24	-5.94
Haematoxylum campechianum	Logwood	-51.47	-28.15	-5.46
Heliotropium angiospermum	Dog's Tail	27.59	0.71	-13.32
Hibiscus rosa-sinensis	Hibiscus	-7.00	-1.63	15.21
Hyptis verticulata	John Charles	-8.63	-43.68	-12.31
Ipomoea carnea	Morning Glory	32.63	19.80	4.85
Ixora spp.	Ixora (White)	-10.89	-13.53	-28.00
Lantana camara	White Sage (Red Flowers)	-23.18	0.79	1.52
Leucaena leucocephala	Leucaena	-15.79	15.10	19.83
Lippia alba	Colic Mint	-339.10	35.10	-1.60
Mangifera indica	Julie Mango	-306.41	-140.07	72.60
Melicoccus bijugatus	Guinep	5.88	3.31	-5.31
Moghana strobilifera	Wild Hops	-18.15	-9.56	-4.28
Momordica charantia	Wild cerasee	-5.30	37.56	2.68
Morinda citrifolia	Noni (Hog Apple)	36.28	2.13	-24.83
Moringa oleifera	Moringa	-7.93	0.00	-1.75
Mucuna pruriens	Cow Itch	-14.88	3.87	-24.65
Musa sapientum	Banana Lacatan	32.13	51.94	44.76
Nerium oleander	Oleander	25.14	24.17	-61.69
Nicotiana tabacum	Tobacco (Donkey Rope)	-9.20	7.99	-37.32
Ocimum micranthum	Wild Barsley	25.89	-15.94	-80.71
Parthenium hysterophorus	Dog-flea Weed	-38.95	-2.34	5.78
Pedilanthus spp.	Monkey Fiddle	-25.12	9.13	-55.50
Peperomia amplexicaulis	Flat Rock/Jackie's Saddle	16.37	14.18	-7.59
Persea americana	Avocardo	-53.13	24.61	-6.40
Petiveria alliacea	Anamu (Guinea Hen Weed)	-60.41	1.69	39.15
Phyllanthus uvinaria	Chamber Bitter	-36.94	12.07	-34.52
Pimenta dioica	All Spice (Pimento)	-70.41	-55.00	-221.28
Piper amalago	Black Jointer	-10.48	0.25	3.12
Pithecellobium unguis-cati	Privet	-3.26	25.91	-9.32
Plectranthus amboinicus	French Thyme	-62.85	-78.26	-68.88
Plectranthus blumei	Joseph's Coat	-6.66	-5.67	-17.21
Psidium guajava	Guava	-38.20	-5.41	8.90
Punica granatum	Pomegranate	-76.33	-15.65	29.91
Rhizophora mangle	Red Mangrove	-1.17	-3.51	9.25
Ricinus communis	Castor Oil	24.65	4.89	-10.54
Rivina humilis	Dog Blood	-4.51	32.69	3.13
Rosmarinus officinalis	Rosemary	31.44	26.12	24.82
Rytidophyllum tomentosum	Search-me-heart	-16.25	-1.42	26.02
Sansevieria spp.	Modda-In-Law Tongue (Yellow)	9.27	12.15	-56.09

Sansevieria spp.	Modda-In-Law Tongue (Green)	-34.06	23.39	-13.53
Satureja viminea	Peppermint	-60.59	-12.61	-19.15
Simarouba glauca	Bitter Damsel/ Bitter Damson	-2.81	8.17	4.56
Solanum torvum	Susumber	-18.84	9.65	5.94
Spigelia anthelmia	Worm Grass	-15.57	37.40	36.03
Syzygium cumini	Ribena	16.02	35.00	24.35
Tecoma stans	Jamaican Lilac	59.23	61.50	57.14
Terminalia catappa	Almond	6.34	7.03	6.64
Thymus vulgaris	Thyme	-23.04	-32.37	-26.48
Wedelia trilobata	Wild Marigold	10.62	0.00	-6.90
Ziziphus mauritinia	Coolie Plum	-75.96	-22.97	8.53

Extracts with Anti-Denaturation Activity ≥ 20%

Table 2: Anti-denaturation activity of crude ethanolic leaf extracts from five Jamaican plants and positive control (aspirin).

		% Anti-Denaturation Activity of Ethanolic Leaf Extracts at Three Concentrations		
Plant Scientific Name	**Plant Common Name**	**0.25 µg/mL**	**0.50 µg/mL**	**1.00 µg/mL**
Cajanus cajan	Gungo	54.46	62.06	62.31
Cinnamomum zeylanicum	Cinnamon	24.18	34.63	49.61
Mangifera indica	Julie Mango	-306.41	-140.07	72.60
Cordia alba	Duppy / Sibble Cherry	65.47	39.53	-86.35
Tecoma stans	Jamaican Lilac	59.23	61.50	57.14
Aspirin (Positive Control)	-	35.30	18.80	21.50

It was observed that crude extracts of both *C. cajan* and *C. zeylanicum* exhibited concentration-dependent inhibition of denaturation of protein, where the inhibition of the denaturation of the BSA increased with the concentration of the extract; the highest anti-denaturation activities were observed at 1.00 µg/ml for both plant extracts (62.31% and 49.61% respectively). While the crude extract of *M. indica* did not exhibit anti-denaturation activity at 0.25 - 0.50 µg/ml concentrations, relatively high inhibition (72.60%) of BSA occurred at 1.00 µg/ml. On the other hand, a concentration-dependent inhibition of denaturation of protein was observed for crude extract of *C. alba* at concentrations 0.25 - 0.50 µg/ml. However, in this instance, the highest anti-denaturation activity was observed at the lowest concentration (65.47% at 0.25 µg/ml). No activity was observed at 1.00 µg/ml. The crude extract of *T. stans* possessed relatively good anti-denaturation activity; however this was not concentration-dependent. Similar observations were made for the reference drug aspirin. Crude extracts of *C. cajan* and *T. stans* were observed to exhibit significantly higher anti-denaturation activities at all three concentrations investigated when compared with the reference drug aspirin.

Fractionation of the crude ethanolic leaf extracts, resulted in the complete loss of anti-denaturation activity in *C. cajan* and *C. alba* (Tables 3 & 4). Varying levels of anti-denaturation activity were observed in fractions 5 and 12 of *C. zeylanicum* and *M. indica*, respectively, and fractions 9, 10, 15 and 16 of *T. stans*. A reverse concentration-dependent inhibition of protein denaturation was observed for fraction 5 of *C. zeylanicum*, with the highest anti-denaturation activity of 41.69% being observed at the lowest concentration of 0.25 µg/ml (Table 5). While this activity was lower, when compared to the crude extract (49.61% at 1.00 µg/ml), it was higher than the 24.18% observed in the crude extract at 0.25 µg/ml concentration (Table 2). There was a significant decrease in the anti-denturation activity of *M. indica* on fractionation when compared with the 72.60% observed at the 1.00 µg/ml concentration in the crude extract; only fraction 12 exhibited activity of 21.53% at 0.50 µg/mL (Table 6). Conversely, fractions 9, 10, 15 and 16 of *T. stans* inhibited the denaturation of protein (Table 7). Of note, is that a reverse concentration-dependent inhibition of protein denaturation was observed in fractions 15 and 16, with the highest anti-denaturation activities being observed at the lowest concentration. In addition, a marked increase was observed in the anti-denaturation activity in fraction 16, which effectively inhibited the denaturation of protein at 70.76 % and 68.93% at 0.25 µg/mL and 0.50 µg/mL respectively, compared with 59.23% and 61.50% at 0.25 µg/mL and 0.50 µg/mL respectively, in the crude extract. In fact, of all the five

extracts investigated, *T. stans* was the only extract that effectively increased the inhibition of protein denaturation across two of the three concentrations investigated, upon column fractionation.

Table 3: Anti-denaturation activity of *C. Cajan* (gungo) leaf fractions.

	% Anti-Denaturation Activity of Gungo Leaf Fractions at Three Concentrations		
Fractions	**0.25 µg/mL**	**0.50 µg/mL**	**1.00 µg/mL**
1	- 5.17	-1.67	1.56
2	-5.17	-1.67	-1.56
3	-1.72	-1.67	-6.25
4	-6.90	-3.33	-7.81
5	-5.17	-5.00	-12.50
6	-7.14	-4.76	-14.49
7	-12.50	-4.76	-27.54
8	-7.14	-4.76	-4.35
9	-17.86	-20.63	-42.03
10	-28.57	-34.92	-59.42
11	-11.76	-23.64	-38.71
12	-9.80	-18.18	-27.42
13	-13.73	-16.36	-20.97
14	-9.80	-16.36	-19.35
15	-13.73	-14.55	-16.13
16	-11.76	-14.55	-30.65

DISCUSSION

Adoption of the BSA protein denaturation assay for the *in vitro* evaluation of anti-inflammatory potential of ethanolic plant extracts circumvented the ethical issues associated with the use of animals, especially in the early stages of screening for plants with potential lead anti-inflammatory compounds. In addition, protein denaturation has been described as a pathological process which involves the loss of configuration, and as a result, loss of functionality. [4] This makes the reduction in protein denaturation, and by extension the BSA protein denaturation assay, ideal for the determination of anti-inflammatory potential. It should be noted that the experiments were conducted at pH 6.4, which represents the pathological pH (6.2 - 6.5) at which, reportedly heat treated BSA is stabilized (denaturation is inhibited) by several NSAIDs, [11] lending further credence to the choice of method.

Various models including, but not limited to carrageenan-induced paw oedema, human red blood cell membrane stabilization, lipoxygenase inhibition, as well as protein denaturation have been used to confirm anti-inflammatory activities of *C. cajan* (Gungo),[17-19] *C. zeylanicum* (Cinnamon),[20-22] *M. indica* (Julie mango) [23 -25] and *T. stans* (Jamaican lilac). [26-28]

Table 4: Anti-denaturation activity of *C. alba* (duppy / sibble cherry) leaf fractions.

	% Anti-Denaturation Activity of Duppy Cherry Leaf Fractions at Three Concentrations		
Fractions	**0.25 µg/mL**	**0.50 µg/mL**	**1.00 µg/mL**
1	-14.49	-15.84	-51.82
2	-13.04	-18.81	-87.59
3	-23.19	-21.78	-86.13
4	2.90	11.88	-113.14
5	18.84	-26.73	-75.18
6	-18.00	-16.26	-18.00
7	-23.67	-34.96	-53.78
8	-35.00	-49.05	-62.17
9	-44.00	-58.27	-73.82
10	-36.00	-38.21	-55.42
11	-11.26	-13.21	-8.79
12	-13.96	-26.04	-11.54
13	-13.96	-26.04	-11.54
14	-13.51	-27.55	-19.51
15	-0.90	-11.70	-3.85
16	-8.76	-11.41	-6.68

Table 5: Anti-denaturation activity of *C. zeylanicum* (cinnamon) leaf fractions.

	% Anti-Denaturation Activity of Cinnamon Leaf Fractions at Three Concentrations		
Fractions	**0.25 µg/mL**	**0.50 µg/mL**	**1.00 µg/mL**
1	-6.63	-7.51	-10.50
2	-9.64	-11.56	-13.26
3	-3.01	-7.51	0.00
4	12.65	1.16	5.52
5	41.69	32.94	31.49
6	-27.06	-20.36	-1.89
7	-46.41	-62.65	-43.40
8	-68.63	-77.11	-49.06
9	-60.78	-75.90	-47.17
10	-45.10	-63.86	-37.74
11	-22.35	-85.11	-103.31
12	-131.76	-275.53	-25.07
13	-190.59	-405.32	-402.48
14	-200.00	-385.11	-314.05
15	-160.00	-203.19	-204.13

Fractions with Anti-Denaturation Activity ≥ 20%

Hence, it was not surprising that the crude extracts of these plants effectively inhibited the denaturation of protein. While there was limited information available with respect to *C. alba* (Duppy cherry) and none related

to its anti-inflammatory activity, the results clearly indicate that this plant has potential as an anti-inflammatory agent, thus making this study even more significant. Being complex mixtures containing multiple compounds, which may interact antagonistically, causing interference or masking the activity of one another, and with the vast majority of their actives being present at low concentrations, crude extracts are usually subjected to various separation and concentration processes. [29] The five crude ethanolic extracts were therefore column fractionated, in an attempt to improve their anti-denaturation activity by way of separation of the compounds to greater purity.

Table 6: Anti-denaturation activity of *M. indica* (julie mango) leaf fractions.

	% Anti-Denaturation Activity of Julie Mango Leaf Fractions at Three Concentrations		
Fractions	**0.25 µg/mL**	**0.50 µg/mL**	**1.00 µg/mL**
1	-8.94	-14.08	-15.21
2	-22.98	-34.30	-58.03
3	-24.26	-41.88	-52.96
4	-27.23	-44.40	-53.80
5	-32.77	-44.40	-58.59
6	-4.78	-15.25	-13.32
7	-15.82	-27.50	-33.81
8	-17.91	-32.50	-27.87
9	-13.84	-11.21	-20.54
10	-31.13	-20.35	-22.97
11	-11.32	-1.47	0.27
12	3.77	21.53	-3.78
13	-15.22	-28.00	-25.41
14	2.98	-15.75	-10.66
15	-11.92	-9.77	-10.48
16	-21.32	-22.50	-21.90

Fractions with Anti-Denaturation Activity ≥ 20%

However, there was complete loss of activity in fractions of *C. cajan* and *C. alba*. This suggests that compounds present in the crude extracts of these two plants may have been acting in synergy and as a result did not exhibit anti-denaturation activity when separated. Indications are that it may be more efficacious to apply the crude extracts of these two plants directly to potential anti-inflammatory formulations. On the other hand, *M. indica*, *C. zeylanicum* and *T. stans* clearly demonstrated anti-denaturation activity on fractionation. Of significance, was that fractions of both *C. zeylanicum* and *T. stans* inhibited the denaturation of protein in a reverse concentration-dependent manner, exhibiting the highest anti-denaturation activities at the lowest concentration; 0.25 µg/mL. This is in keeping with observations made by Williams *et al.*, [11] and also strengthens indications by Grant *et al.*, [30] that one of the features of non-steroidal anti-inflammatory drugs was the stabilization of BSA (inhibition of protein denaturation) at concentrations < 1.00 µg/mL.

Table 7: Anti-denaturation activity of *T. Stans* (Jamaican lilac) leaf fractions.

	% Anti-Denaturation Activity of *Tecoma Stans* Leaf Fractions at Three Concentrations		
Fractions	**0.25 µg/mL**	**0.50 µg/mL**	**1.00 µg/mL**
1	-17.05	-6.96	-11.59
2	-32.78	-26.94	-19.18
3	-59.27	-39.29	-32.18
4	-78.81	-53.75	-45.18
5	-93.71	-62.94	-58.18
6	-17.86	-30.30	-20.77
7	-71.43	-43.94	-13.66
8	-25.00	-31.82	3.28
9	0.00	10.61	44.81
10	0.00	50.00	54.64
11	-19.49	-12.08	-7.73
12	-15.25	-10.07	-2.98
13	3.39	-3.36	0.60
14	18.64	6.04	5.95
15	47.71	31.61	23.81
16	70.76	68.93	51.49

Fractions with Anti-Denaturation Activity ≥ 20%

Although the mechanism responsible for the anti-denaturation property of BSA has not been confirmed, results from 1D proton NMR BSA-compound interaction analyses have suggested that BSA has two main binding sites on the aliphatic threonine and lysine residue regions resulting in interaction signals at about 3.0-3.8 ppm, and on its aromatic tyrosine rich region with interaction signals at about 6.3 – 7.8 ppm. It was on this basis, predicted that therapeutic molecules could therefore be activating the tyrosine motif rich receptor dually with threonine, which regulates signal transduction biological pathways for their overall biological action. [31-33]

CONCLUSION

The study clearly demonstrates that the ethanolic extracts of *Cajanus cajan*, *Cinnamomum zeylanicum*, *Cordia alba*, *Mangifera indica* and *Tecoma stans* effectively inhibited the denaturation of BSA *in vitro*, in a manner comparable to, and even more significant than the reference anti-inflammatory drug, aspirin. It can therefore be concluded, that these extracts possess significant anti-inflammatory activity. In addition, indications are that the direct use of crude ethanolic leaf extracts of *C. cajan* and *C. alba*, may be more efficacious in anti-inflammatory formulations than isolated

compounds. Further investigations of the fractions of *C. zeylanicum*, *T. stans* and *M. indica* will however be necessary, in order to isolate potential lead anti-inflammatory compounds.

Acknowledgements: The authors would like to acknowledge contributions made by Dr. Melaine Randle and Ms. Channtal Golding who ably assisted with the editing of this manuscript. The project was funded by the Government of Jamaica.

REFERENCES

1. Kandikattu, K., Bharath, RKP, Venu, PR, Sunil, KK, Ranjith Singh, BR. Evaluation of anti-inflammatory activity of *Canthium parviflorum* by *in-vitro* method. Indian J. Res. Pharm. Biotech. 1(5), 2013, 729-730.
2. Dharsana, JN and Mathew, SM. Preliminary screening of anti inflammatory and antioxidant activity of *Morinda umbellata*. Int. J. Pharm. Life Sci., 5(8), 2014, 3774-3779.
3. Anyasor, GN, Funmilayo, O, Odutola, O, Olugbenga, A, Oboutor, EM. Evaluation of *Costus afer Ker Gawl*. *in vitro* anti-inflammatory activity and its chemical constituents identified using gas chromatography-mass spectrometry analysis. J. Coastal Life Med., 3(2), 2015, 132-138.
4. Sridevi, G, Sembulingam, K, Muhammed, I, Srividya, S, Prema, S. Evaluation of *in- vitro* anti-inflammatory activity of *Pergularia daemia*. World J. Pharm. Res., 4(6), 2015, 1100-1108.
5. Nargund, SL, Murugan, V, Nargund, LVG. Hrishekeshavan, HJ. Evaluation of *in-vitro* anti-inflammatory activity of novel substituted thienol [2,3-*d*]pyrimidin-4-yl-amine molecules. Ultra Scientist, 28(1), 2016, 11-16.
6. Chatterjee, P, Chandra, S, Dev, P, Bhattacharya, S. Evaluation of anti-inflammatory effects of green tea and black tea: A comparative *in vitro* study. J. Adv. Pharm. Technol. Res., 3(2), 2012, 136-138.
7. Murugesan, D, Deviponnuswamy, R. Potential anti-inflammatory medicinal plants – A Review. Int. J. Pharm. Pharm. Sci.,6(4), 2014, 43-49.
8. Tatti, PN, Anitha, S, Shashidhara, S, Deepak, M, Bidari, S. Evaluation of *in-vitro* anti-denaturation activity of isolated compound of *Butea monosperma* Bark. Pharma Sci. Monitor, 3(4), 2012, 2314-2320.
9. Dar, SA, Yousuf, AR, Ganai, FA, Sharma, P, Kumar, N, Singh, R. Bioassay guided isolation and identification of anti-inflammatory and anti-microbial compounds from *Urta dioica* L. (Urticaceae) leaves. Afr. J. Biotechnol., 11(65), 2012, 12910-12920.
10. Khuda, F, Iqbal, Z, Khan, A, Zakiullah, Shah, Y, Ahmad, L, Nasir, F, Hassan, M, Ismail, Shah, WA. Evaluation of anti-inflammatory activity of selected medicinal plants of Khyber Pakhtunkhwa, Pakistan. Pak. J. Pharm. Sci., 27(2), 2014, 365-368.
11. Williams, LAD., Connar, AO, Latore, L, Dennis O, Ringer S, Whittaker, JA, Conrad J, Vogler B, Rosner H, Kraus, W. The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) Bovine Serum Albumin (BSA) is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals in the early stages of the drug discovery process. West Indian Med. J., 57(4), 2008, 327-331.
12. Ramalingam, R, Madhavi, BB, Nath, AR, Duganath, N, Sri, EU, Banji, D. *In vitro* anti-denaturation and antibacterial activities of *Zizyphus oenoplia*. Der Pharmacia Lettre, 2(1), 2010, 87-93.
13. Asprey, GF, Thornton, P. Medicinal plants of Jamaica, Part I – IV. West Indian Med. J., 2, 1953-1954, 233-352.
14. Ayensu, E. Medicinal Plants of the West Indies. Reference Publications, INO, Algonac, Michigan, 1981**,** pp.282.
15. Drăgan, M, Stan, CD, Pânzariu, A, Profire, L. Evaluation of anti-inflammatory potential of some new ferullic acid derivatives. Farmacia, 64(2), 2016, 194-197.
16. Wong, TM, Robinson, M, Hibbert, S, Williams, LAD. Isolation of anti-inflammatory compounds from Musa sapienttum (Banana) leaf extracts using the anti-denaturation of bovine serum albumin (BSA) assay. In: *In-vitro* Bioassays and Chromatographic Analyses Used to Screen Natural Products from Jamaica in the 21st Century. Cheryl E. Green, Ed., Research Signpost, Trivandrum, Kerala, India, 2015, 33-47.
17. Jaliwala, YA, Mohanti, PK, Kumar, JN. Antiulcer and anti-inflammatory activity of *Cajanus cajan* Linn. Int. Res. J Pharm., 5(4), 2014, 313-316.
18. Mueller, M, Puttipan, R, Janngeon, K, Unger, FM, Viernstein, H, Okonogi, S. Bioactivities of the Thai medicinal and edible plants *C. cajan*, *M. citrifolia* and *O. americanum*. Int. J. Pharm. Pharm .Sci., 7(10), 2015, 237-240.
19. Panda, OP., Naidu, PS, Saraswathi, B. Phytochemical screening and *in-vitro* anti-inflammatory activity of *Cajanus cajan*. Int. J. Current Res. Health Biol. Sci., 1(4), 2016, 202-207.
20. Manosi, D, Suvra, M, Budhimanta, M, Jayram, H. Ethnobotany, phytochemical and pharmacological aspects of *Cinnamomum zeylanicum* Blume. Int. Res J. Pharm., 4(4), 2013, 58-63.
21. Joshi, K, Awte, S, Bhatnagar, P, Walung, S, Gupta, R, Joshi, S, Sabharwal, S, Bami, S, Padalkar, AS. *Cinnamomum zeylanicum* extract inhibits proinflammatory cytokine TNF : *in vitro* and *in vivo* studies. Res. Pharm. Biotechnol., 2(2), 2010, 14 -21.
22. Mitul, P, Handral, M. Anti-myotoxic and anti-inflammatory activity of *Cinnamomum zeylanicum* extracts against Naja kaouthia snake venom. Indo Amer. J. Pharm. Res., 3(6), 2013, 4226 - 4237.
23. Sarkar, M. Evaluation of the anti-inflammatory activity of methanolic leaf extract of *Mangifera indica* L. (Anacardiaaceae) in rats. Int. J. Drug Dev. Res., 7(3), 2015, 21-25.
24. Mohan, GL, Deepak, M, Viswanatha, GL, Savinay, G, Hanumantharaju, V, Rajendra, CE, Praveen, HD. Anti-oxidant and anti-inflammatory activity of leaf extracts and fractions of *Mangifera indica*. Asian Pac. J. Trop. Med., 6(4), 2013, 311-314.
25. Madduluri, S, Silaran, B, Balasekharan, C. *In vitro* evaluation of anti-inflammatory activity of methanolic and ethanolic leaf extracts of five indigenous plants in South India. Int. J.Pharm. Tech. Res., 6920, 2014, 569-574.

26. Kameshwaran, S, Suresh, V, Arunachalan, G, Royal Frank, P, Manikandan, V. Evaluation of antinociceptive and anti-inflammatory potential of flower extract of *Tecoma stans*. Indian J. Pharmacol., 44(4), 2012, 543-544.

27. Dash, S, Das, C, Sahoo, DC, Sahoo, AC. Phytochemical composition, anti-inflammatory and analgesic activities of *Tecoma stans* Linn (Bignoniaceae). Nature Pharm. Technol., 1(2), 2011, 5-8.

28. Prajapathi, DD, Patel, NM. *In-vitro* anti-arthritic activity of *Tecoma stans* (Linn.) leaves. Algerian J. Nat. Prod., 3(2), 2015, 153-158.

29. Nwodo, UU, Ngene, AA, Iroegbu, CU, Obiiyeke, GC. Effects of fractionation on antibacterial activity of crude extracts of *Tamarindus indica*. Afr. J. Biotechnol., 9(42), 2010, 7108-7113.

30. Grant, NH, Alburn, HE, Kryzanauska, C. Stabilization of serum albumin by anti-inflammatory drugs. Biochem. Pharmacol., 19, 1970, 715-722.

31. Williams, LAD, Vasquez, EA, Milan, PP, Zebitz, C, Kraus, W. *In vitro* anti-inflammatory and anti-microbial activities of phenylpropanoids from *Piper betle* Piperaceae. In: Proceeding of the Phytochemical Society of Europe: Natural Products in the New Millennium: Prospects and Industrial Application; A. P. Rauter, F. B. Palma, J. Justino, M. E. Araujo, and S. P. Santos, (Eds.) Kluwer Academic Publisher, Dordrecht, The Netherlands, 74, 2002, 221-227.

32. Williams, LAD, Rösner, H, Conrad, J, Möller, W, Beifuss, U, Chiba, K, Nkurunziza, JP, Kraus, W. Selected secondary metabolites from the Phytolaccaceae and their biological/pharmaceutical significance. In: Recent Research Developments in Phytochemistry, Research Signpost, Trivandrum, Kerala, India, 6, 2002, 13 - 68.

33. Rösner, H, Williams, LAD, Jung A, Kraus, W. Disassembly of microtubules and inhibition of neurite outgrowth, neuroblastoma cell proliferation, and MAPKinase tyrosine-dephosphorylation by dibenzyltrisulphide. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research, 1540(2), 2001, 166 – 177.

Source of Support: Nil, **Conflict of Interest:** None.