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SHORT COMMUNICATION

PHARMACOLOGICAL EVALUATION OF *Alstonia scholaris* Linn R. Br. FROM PAPUA NEW GUINEA

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Alstonia scholaris Linn. R. Br. (Apocynaceae) is a large evergreen tree commonly distributed throughout the lowlands of PNG and is an inhabitant of primary and secondary forests, lower montane rainforests, monsoon forests and savannah woodlands [1]. It is used as a traditional medicine in many parts of PNG for the treatment of severe fevers including malarial fever, and other conditions such as diarrhoea, dysentery, headaches, stomach ache, cough, shortness of breath (SOB), TB, pain and as an oral contraceptive [2]. In other parts of the world, it is used as febrifuge and tonic to aid digestion and stimulate appetite, as remedy for liver and intestinal problems, for treatment of chronic malaria with enlarged spleen, against diarrhoea and as wash for haemorrhoids; as anti-diabetic, anti-helminthic and anti-epileptic agents [2, 3]. *A scholaris* has been reported to contain numerous Indole Alkaloids [4-6], some major ones being echitamine, akuammigine and akuammicine [4], Manilamine, N^4-methyl angustilobine B, Angustilobine B N^4-oxide, 19, 20-(*E*)-vallesamine, 20S-Tubotaiwine and 6,7-seco angustilobine B [7]. Triterpenoids [8,9,10], Flavonoids and Glycosides [10,11] and beta-sitosterol (a phytosterol) [10].

Some of its reported pharmacological activities include Anti-anxiety [12], Anti-plasmodial [13], Immuno-stimulating effect [14], Anti-cancer [15], Hepato-protective [16], Anti-nociceptive [17], Anti-inflammatory [17] and Anti-ulcerogenic [18]. Toxicity studies on the acute and sub-acute effects in mice and rats have revealed dose dependant effects [19].

The pharmacological evaluation of *A. scholaris* comprises one aspect of a project on standardization of herbal medicine derived from *A. scholaris.* Standardization ensures that the preparation is stable, has correct potency and is reproducible when administered by the herbal practitioner within the settings of the traditional use of the plant. Hence, it is essential in verifying that the plant elicits the activity for which it is used traditionally by subjecting extracts of the leaves and bark of the plant from various locations in PNG to the following bioassays: (a) Cytotoxicity, (b) Nitric oxide inhibition, (c) PGE2 inhibition, and (d) Phagocytosis. Sites of sample collection included Brown River in Central, Losuia in Kiriwina, Taurama campus of University of PNG in NCD and Lorengau in Manus.

Cytotoxicity effect of *A. scholaris* extracts was carried out on Mouse lymphoblast (P388), Mouse Swiss albino embryonic fibroblast (3T3) and Mouse leukemic monocytes macrophage (RAW) cell lines. P388 (mouse lymphoblast) was used mainly as an initial screen for general toxicity from which a base-line concentration was determined for carrying out the other assays. In general, results from this assay indicated relatively high percentage (%) inhibition of P388 cells by leaf water extracts at 200ug/mL in comparison to the bark extracts in water. The un-dried leaf extract from Taurama, displayed the highest activity at the same concentration. 200ug/mL of the methanol extract of un-dried leaves from the same location also showed the highest inhibition against P388 cell line relative to other locations in the study.

Additionally, values for the concentration at which 50% of the activity is inhibited (the IC 50) were determined using the methanol extracts with a concentration of 200ug/mL, against the mouse lymphoblast cells. IC50s ranged from 120.25 to 408.19ug/mL, indicating that all dried leaf and bark extracts had relatively high IC_{50} and hence low toxicity as compared to one of the positive controls, Curcumin, having an IC50 of 15.55ug/mL. Moreover, percentage inhibition of 3T3 and RAW cells revealed the dried leaf extract in methanol from Lorengau (Manus Province) as having the highest inhibitory effect against both cell lines. Leaf extracts displayed relatively higher inhibition against 3T3 cells than the bark extracts. RAW cells seemed to show relatively greater sensitivity to both leaf and bark extracts at the concentration assayed.

Results from the PGE_2 inhibition assay showed that all aqueous leaf and bark extracts of *A. scholaris* from all the study sites displayed no inhibitory effect on PGE_2 production as compared to the positive

inhibitor control. With respect to phagocytosis activity, water and methanol extracts of the un-dried and dried leaves of the tree from the Taurama campus revealed that the aqueous extract of un-dried leaves had the highest positive phagocytic activity in human blood. Methanol extracts had the lowest phagocytic effect.

The water extracts of un-dried leaves and bark of *A. scholaris* from Losuia and Brown River exhibited phagocytic activity however, the bark had relatively higher activity for both locations. The water extracts of un-dried leaves and bark from Lorengau displayed positive phagocytosis with the latter displaying relatively higher activity.

In conclusion, pharmacological evaluation of *A. scholaris* extracts has to some extent provided scientific rationale for its traditional use in the treatment of fever. Water extracts displayed less inhibitory effect on cell growth at 200ug/mL relative to methanol extracts at the same concentration however, IC_{50} values of the latter revealed relatively high values as compared to one of the controls (curcumin). Methanolic and aqueous extracts prepared through boiling, did not possess any inhibitory effect on PGE_2 production in the initial experiments. Un-dried leaf and bark extracts in hot water demonstrated positive phagocytosis activity wherein the bark extracts from all locations exhibited relatively high activity (approx. 30% increase). Hence results of the bioassays indicate that the fever-reducing property of *A. scholaris* is most probably propagated through phagocytosis, the innate component of the body's defence system. It is recommended that the un-dried bark extract from Taurama campus be subjected to phagocytosis assay since it was not included in the initial assay. Establishment of a thin layer chromatographic profile for the extract exhibiting the highest relative phagocytosis activity can be utilized as a reference against which extracts of *A. scholaris* from the other locations can be compared.

REFERENCES

1. Woodley E (ed)., Medicinal Plants of Papua New Guinea, Part 1: 1991; Morobe, Wau Ecological Institute Handbook No. 11, 17
2. Medicinal Plants in Papua New Guinea, published by the WHO Western Pacific Region 2009
3. Kam. T. S, Nyeoh. K. T, Sim. K. M, Yoganathan. Y, Alkaloids from Alstonia scholaris, Phytochemistry, 1997, 45 (6): 1303 – 1305
4. Salim. A. A, Garson. M. J, Craik. D. J, New indole alkaloids from the bark of Alstonia scholaris, Journal of Natural Products, 2004, 67 (9):1591 – 1594
5. Cai. X-H, Du. Z-Z, Luo. X-D, Unique monoterpenoid indole alkaloids from Alstonia scholaris, Organic letters, 2007, 9 (9): 1817 – 1820

6. Zhou. H, He, H. P, Luo. X. D, Wang. Y. H, Yang. X. W, Di. Y. T, Hao. X. J. H, Three new alkaloids from the leaves of Alstonia scholaris, Helvetica Chimica Acta, 2005, 88 (9): 2508 – 2512
7. Macabeo et al.; Indole alkaloids from the leaves of Philippine Alstonia scholaris; Phytochemistry 66, 2005; pp 1158 – 1162
8. Wang. F, Ren. F-C, Liu. J-K, Alstonic acids A and B, unusual 2,3 – secofernane triterpenoids from Alstonia scholaris, Phytochemistry, 2009, 70: 650 – 654
9. Shetty P, Mangaokar, Sane R; HPTLC Determination of Ursolic Acid in Alstonia scholaris R.Br; Journal of Planar Chromatography vol 20 2007 1; pp 68 – 68
10. Hemalatha. K, Satyanarayana. D, Phytochemical constituents of root bark of Alstonia scholaris R. Br, Asian Journal of Chemistry, 2008, 20 (7): 5405 – 5408
11. Thomas S, Kanaujia A, Ghosh D, Duggar R, Katiyar C; Alstonoside, a secoiridoid glucoside from Alstonia scholaris ; Indian J. of Chemistry vol. 47B, 2008, pp 1298-1302
12. Arulmozhi. S, Papiya. M. M, Vishnu. A. K, L. Sathiya Nanayan, Prasad. T, Anti-anxiety activity of Alstonia scholaris Linn. R.Br., Pharmacology online 2008, 3: 761 – 775
13. Keapradub. N, Kirby. J. C, Steele. J. C. P, Houghton. P. J, Antiplasmodial activity of extracts and alkaloids of three Alstonia species from Thailand, Planta Medica, 1999, 65 (8): 690-694
14. Iwo. M. I, Soemardji. A. A, Retnoningram. D. S, Sukrasno, U. Uu Mar, Immunostimulating effect of Pule (Alstonia scholaris L.Br., Apocynaceae),Clinical Hemorheology and Microcirculation, 2000, 23 (2-4): 177 – 183
15. Saraswathi. V, Mathuram. V, Subramanian. S, Govindasamy. S, Inhibition of glycolysis and respiration of sarcoma-180 cells by echitaminechloride, Chemotherapy, 1998, 44 (33): 198-205
16. Lin. S-C, Lin. C-C, Lin. Y-H, Supriyatna. S, Pan. S-L, The protective effect of Alstonia scholaris R.Br. on hepatotoxin-induced acute liver damage, American Journal Chinese Medicine,1996, 24 (2): 153-164
17. Arulmozhi. S, Papiyer. M. M, Purnima. A, Basavaraj. H, L. Sathiya Narayan., Antinociceptive and Anti-inflammatory Activities of Alstonia scholaris Linn. R.Br. Pharmacognosy Magazine, 2007, 3 (10): 106-111
18. Arulmozhi. S, Papiya. M. M, Purnima. A, L. Sathiya Narayan, Pharmacological activities of Alstonia scholaris Linn. (Apocynaceae) – A Review, Pharmacognosy Reviews 1, 2007, (1): 163 – 170
19. Baliga. J. S, Jagetia, G, C., Uloor, J.N., Baliga. M.P., Venkatesh. P., Reddy, R., Rao, KVNM., Baliga, B.S., Devi, S., Raju, S., Veeresh,V., Reddy, T.K., Bari, K.L., The evaluation of the acute toxicity and long term safety of hydroalcoholic extract of Sapthaparna (Alstonia scholaris) in mice and rats, Toxicology Letters, 2004, 151 (2): 317 – 326