Determination Of The Presence Of Flavonoids In The Leaves, Seeds And Branches Of The Matured Plant, Indigofera Arrecta.

Doreen Amponsah, Dr. Henry Nagai

Abstract: The extracts were concentrated to obtain a mass of 0.9g, 1.2g, 1.4g of seeds, leaves and branches respectively. After the separation of the pigment into hexane, the mass of the residue obtained were 0.7g for seeds, 0.9g for leaves and 1.0g for branches. The Cyanidin's test for the various masses of the extracts of the plant materials after washing with hexane gave the evidence of the presence of flavonoids. The number of components could not be obtained from the eluates of the column chromatography, because there were no T.L.C materials available. But when the component of each plant material was subjected to cyanidin's test, it gave the proof of the presence of flavonoid.

Keywords : Indigofera arrecta, Cyanidin's test, Concentration, Seeds, Leaves, Extracts, Flavonoid**.**

1 INTRODUCTION

Indigofera arrecta Hochst ex. A. Rich1 (Papilionaceae) is a plant which belongs to the family Leguminoceae. It is a common roadside shrub which grows wild in savannah. It is common in the Accra plains, Djelu-Koffe near Keta and Kpeve in Togo. It can also be located in Senegal through Nigeria and all over Tropical Africa, Saudi Arabia, Eastern and Southern Africa also experienced the growth of this plant. Indigofera arrecta is known as Batamau in Hausa, Klenme in Ga and Lademadui in Ewe. It is a small shrub or a woody herb of up to 2m high with grooved grey pubsecent stem. The leaves are pinnate, glaucous, turning blackisk on drying. The leaflets are usually eleven or more. The plant has straight fruits, about six seeded. The fruits are also less than 2cm long, brown and nearly glabrous. It can well withstand drought because it is deeply rooted and is useful as green manure and cover crop. Indigofera arrecta has numerous uses which include medicinal ones[2]. The dried extract of the plant is antiseptic and astringent and has been sprinkled on ulcer. It has been used as s remedy for hysteria and epilepsy. The powdered extract has been mixed with honey and administered for the enlargement of the liver and the spleen, and for nervous disorders.The extract has been applied to reduce swellings in the body, to relieve the bite and sting of venomous insect and reptiles and to soothe burns and scalds.

- *Doreen Amponssah is a lecturer of Department of Cosmetology, Wa Polytechnic,Wa and PhD student of Kwame Nkrumah University of Science of Technology, Ghana.*
  Email: doreenopokuamponsah@gmail.com
- *Dr. Henry Nagai is Country Director Family Health International, Ghana.*

It is also mixed with castor oil and applied to the anal to promote the action of the bowel in children. Mixed with warm water, its application to the pubes and hypogesfrum is said to stimulate the bladder in retention of urine. The juice from the peeled roots mixed with fats is also used as a local application for itching of the skin. In East Africa, a leaf infusion is used for dyeing gourds. The root is also used for colic. In Centre for Scientific Research into Plant Medicine, C.S.R.P.M, Mampong – Akwapim, Ghana; the aqueous extract of the leaves is administered in the treatment of diabetes type (II), maturing onset; which occurs in people over forty years of age, and who may have been normal or diabetic when younger. The extract is sort to control the disease by activating the hormone insulin in the conversion of glucose to glycogen in the body. In light of its numerous and extensive uses and easy availability all over Africa, it is seemingly right to subject the plant to investigation. It was decided as a step in this direction to determine the chemical constituents which could be isolated in a pure state.

1.1 Phytoconstituents

Indican (1) is a characteristic product of the genus Indigofera,3 and has been isolated from Indigofera arrecta and Indigofera tinctoria. Indican, a dried extract of the plant, owes it dyeing properties to the presence of Indigotin (4), a dark blue powder. Indicant is hydrolysed by enzyme emulsion3 to glucose (2) and indoxyl (3), and the latter on exposure to air undergoes oxidation to indigotin. Welmer's3 analysis on the leaf noted that tannins were absent. In 19064 Perkin et al isolated a flavonoid from the dried leaves of Indigofera arrecta after extraction with petroleum ether of the ethanol extract and identified as 3, 7 dirhamnoside of Kaempferol. In 1907 another compound5 was again isolated after extraction with petroleum ether of the acetone extract and identified as indoxyl-β-D-glucose which was found to be identical with indican. The objective of this investigation is to determine whether flavonoids are present in the leaves, seeds and branches of Indigofera arrecta and isolate them

2.0 MATERIALS AND METHODS

The seeds, leaves and branches of the matured plant, Indigofera arrecta, were collected from Centre for Scientific into Plant Medicine (C.S.R.P.M), Mampong-Akwapim.

2.1 Plant treatment
The plant materials were sorted out individually. The plant materials were then air dried for some weeks. They were milled to coarsely powder using a miller.

2.2 Extraction
7.0g of seeds, 7.3g of leaves and 7.5 of branches in their powdered form were refluxed for two hours each, with methanol as extraction solvent in a soxhlet apparatus. The extracts were concentrated in a rotary evaporator and put into a desiccator. After, the extracts were washed with hexane in a separatory funnel and the lower layer was drained into an Erlenmeyer flask. This lower layer contains the pigment.

2.3 Cyanidin's test
45ml of 80% methanol was added to 5ml of the sample which was allowed to mix thoroughly. 2ml of the mixture each was put into two clean test tubes. One test tube was reserved to serve as a blank control. 0.5ml of concentrated hydrochloric acid was added to the other test tube followed by addition of magnesium turnings.

3.0 RESULTS AND DISSCUSION

Cyanidin's test on:	Observation	Inference
Leave extract	Pink colour formed	Flavonoid present
Seed extract	Pink colour formed	Flavonoid present
Branch extract	Pink colour formed	Flavonoid present

3.1 Column chromatography
A column of length 60cm and internal diameter 3cm was cleaned with detergent and dried. The column was wet packed as follows: a small cotton wool was used to block the outlet to prevent the flow of silica gel. The silica gel was then packed on it to a height of about 40cm. The mixture of concentrated methanol extract and silica gel was stirred and dried on water bath for some time. The mixture was packed on top of the silica gel followed by cotton to form a protective layer. The column was then eluted using various solvent systems ranging from non-polar to less polar. The eluents used are written below;

(i) Ethylacetae
(ii) Ethylacetate – methanol (19:1)
(iii) Ethylacetate – methanol (10:1)
(iv) Ethylacetate – methanol (8:1)

Only one component was found for each of the plant materials. The components were subjected to yaniding's test and they all confirmed the presence of flavonoids.

4.0 RESULTS AND DISSCUSION
The individual plant materials in their powdered form were refluxed with methanol for 2hours each. The extracts were concentrated to obtain a mass of 0.9g, 1.2g, 1.4g of seeds, leaves and branches respectively. After the separation of the pigment into hexane, the mass of the residue obtained were 0.7g for seeds, 0.9g for leaves and 1.0g for branches. The Cyanidin's test for the various masses of the extracts of the plant materials after washing with hexane gave the evidence of the presence of flavonoids. For the column chromatographic analysis, Ethylacetate was used to elute the sample in the column, which brought out pink – red coloured eluates. It was repeated until the intensity of the colour diminished. This was followed by Ethylacetate – methanol (19:1), which gave one component for each plant material. The polarity of the system was increased by using ethylacetate – methanol (10:1). This also gave one component for each plant material. The column was eluted again using ethylacetate – methanol (8:1) which has a higher polarity than the former. Only one component was obtained for each plant material. The number of components could not be obtained from the eluates of the column chromatography, because there were no T.L.C materials available. But when the component of each plant material was subjected to cyanidin's test, it gave the proof of the presence of flavonoid.

5.0 CONCLUSION
Investigations showed that, the components from the seeds, leaves and branches of the plants Indigofera arrecta contained flavonoid.

6.0 RECOMMENDATION.
Flavonoids are powerful antioxidants with anti-blood clotting properties. It is believe their positive effects on countering oxygen caused damage in the body such as fatty tissue deposits can help avoid heart problems, promote better circulation and overall better health as free radicals in the body are eliminated. There are over 4000 flavonoids found in such places as tea, fruit, vegetables and red wine. Eat more locally grown foods and vegetables to promote your health, renew your strength and prevent diseases.

ACKNOWLEDGEMENT
The authors wish to thank all those who helped for this project to be done.

References
[1] J.B. Harbone, 'Comparative Biochemistry of Flavonoids', p.161. Academic Press, London, 1997.

[2] Walt and Breyer-Bradwigle, 'The Medicinal and Poisonous Plant of Southern and Eastern Africa', p. 613-614. Edinburgh. 1982.

[3] S. Perkin and T. Bloxam, 'J. Chemical Society 91', 1715. 1997.

[4] F. R. Irvine 'Woody Plants of Ghana', p. 84. Oxford University Press, 1991.

[5] N. Bartlett (Ed), 'McGreens-Hill Encyclopedia of Science and Technology', 6th Ed (7, FAB-GEN). p. 136-140.

McGreen-Hill Book Company, New York, 1992.

[6] J. R Corbett. 'The Biochemical Mode of Action of Pesti cides', p.130. Academic Press, New York. 1984.

[7] F. Matsumura, Toxicity of Insecticides', p.240. Plenum Press, New York. 1985.

[8] J.M.P. Martinez, R.B. Llavori, M.J.A. Cabo, and T.B. Pedersen, "Integrating Data Warehouses with Web Data: A Survey," *IEEE Trans. Knowledge and Data Eng.*, preprint, 21 Dec. 2007, doi:10.1109/TKDE.2007.190746.(PrePrint)