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Evaluation of phytochemical and elemental contents of *Haematostaphis barteri* leaves and stem bark in Hong local government area of Adamawa state, Nigeria

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ABSTRACT

Phytochemical and elemental constituents of leaves and stem bark of Haematostaphis barteri was determined in ten locations in Hong Local Government Area of Adanawa state. Quantitative methods were used for the phytochemical determination. Tannins and Saponins were observed in the entire samples investigated. Alkaloids were present in the leaves while flavonoids were recorded in the stem bark. Glycoside was not observed in the samples by the method used. The elemental compositions were determined by Atomic Absorption Spectroscopy (AAS). Essential elements (Na, K, Mg and Ca) and heavy metals (Cu, Zn, and Cd) were recorded in the samples observed. There was no significance difference ($p \leq 0.05$) in terms of essential elements and heavy metal contents among the 10 locations investigated. The phytochemicals observed have many pharmacological properties while the essential elements are much required by human bodies for maintenance of good health.

Key words: Determination, element, leaves, phytochemical, stem bark

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INTRODUCTION

Haematostaphis barteri popularly known as blood plum is a member of anacardiaceae family. The Hausa name is Jinin kafiri. It is found wild in Adamawa and Borno states of Nigeria. The fresh tender leaves are edible. The fruit has oily seed which is edible [1]. *Haematostaphis barteri* is composed of 54.5% oil, 4.11% crude protein, 2.4% crude fibre, 1.0% ash, 9.0% moisture and 29.0% carbohydrate.

In Nigeria and other developing countries, as a result of food shortage and high cost of cultivated green leafy vegetables, wild and semi-wild food resources are frequently consumed as the dominant source of leafy vegetables especially in the rural communities [2]. These plant vegetables play an important role in every day cooking especially in the rural areas. In addition, the vegetables supply calories and nutrients during dry season when there is shortage of cultivated green vegetables and other food resources [3].

Throughout the years, even in the absence of famine, edible plants play an important role in every day cooking. A study carried out by Humphery *et al.*, [4] showed that a variety of leaves are used as seasonings and in sauces at every meal in Southern Niger. They found that the midday meal often centres on cooked wild leaves from *Corchorus tridens, Leptadenia hastate, Hibiscus sabdarifa and Moringa oleifera* flavoured with groundnuts. The side-dish of soup at the evening meal relies on gathered leaves for its flavour. Leaf-bundle snacks are made as well from variety of leaves.

Until recently, little attention has been given to the role of plant foods [5]. By learning more about the protein, fat and mineral content of each plant, one can better assess their importance in nutritional well-being of the communities. The ability to inform nutritionists and public health workers as to which plants can offer the population these nutrients is important.

The nutritional content of vegetables varies considerably though they contain a small proportion of proteins and fats and a relatively high proportion of vitamins, provitamins, dietary minerals, fibres and carbohydrates [6]. Many vegetables also contain phytochemicals which may have antioxidant, antibacterial, antifungal, antiviral and anticarcinogenic properties [7]. Phytochemicals have the capacity to modulate one or more metabolic processes that result in the promotion of better health [8]. Phytochemicals give plants natural defense against diseases and they perform similar function for humans. It is most common to find mixtures of phytochemicals within a plant food. Some plant foods have nutritional and medicinal properties because of the interaction between their nutrients and phytochemical constituents [9].

Several studies have reported elemental contents in plant extracts which are consumed as herbal health drink or in orthodox medicine. The quantitative estimation of various trace elements concentration is important for determining the effectiveness of medicinal plants in treating various diseases and also to understand the pharmacological action. Trace metals are metals in extremely small quantities that reside in plants cells or tissues. They are a necessary part of good nutrition, although they can be toxic if ingested in excess quantities [10]. Toxic metals bio-accumulate in the body via food chain. Reports have shown that, vegetables grown rich heavy metal soils are also contaminated [11]. Vegetable take up metals from contaminated soil through the roots and incorporated them into the edible part of the plant tissues or as a deposit on the surface of vegetables [12].

Diet-related non communicable diseases like obesity, diabetes, hypertension, cardiovascular diseases are increasingly becoming public health problems in Nigeria. This is partly due nutrition transition, which essentially has to do with changes in food consumption patterns associated with modernization, urbanization, economic development and market globalization [9]. There is a shift away from the traditional foods towards overconsumption of processed foods high in saturated fats and sugar but low in fiber, vitamins, phytochemicals and minerals. Some traditional foods like green leafy vegetables contain a lot of promoting bioactive substances. There is contrasting information on some nutritional status of some many vegetables in the tropics [13,14]. The fight against malnutrition and undernourishment continues to be a basic goal of development and a variety of strategies are being applied. The strategy on nutrient-rich food like vegetable is considered essential. Because of this need, the researcher was motivated to analyze some elements (Na, K, Ca, Mg, Cu, Zn, Pb and Cd) and phytochemicals (Alkaloids, Tannin, Saponins, Flavonoids and Glycosides) in Haematostaphis barteri leaves and stem bark in Hong Local Government area of Adamawa State.

EXPERIMENTAL SECTION

Sampling and Sample Preparation

Sampling

Sampling was done according to the method of Ayoola *et al*., [15]. Fresh tender leaves and the stem bark of *Haematostaphies barteri* were collected from 10 locations (Pella, Hong, Hildi, Uba, Gashala, Gaya, Garaha, Mugili, Fa'a and Pubba) in April, 2012 in Hong Local Government area of Adamawa State, Nigeria. Each plant parts were dried under shade.

Sample Preparation

Each dried sample was ground to powder and sieved. Representative samples were obtained from each sample by coning and quartering techniques as described by Crosby and Patel, [16]. This method involves making a cone shape of the Sample, flattened it and divides it into four equal parts; take the opposite two quarters and discard the other two quarters. This was repeated until the sample was reduced to the size required for final analysis and stored in an air tight container.

Sample digestion
The digestion process was carried out as described by Onwunka [17] as follows:

3.0g of each of the powdered sample was weighed and pre-treated with 20 ml nitric acid and allowed to stay overweight. 10 ml perchloric acid was added and heated gently, then vigorously until clear solutions were obtained. The solutions were allowed to cool and then transferred to 100 ml volumetric flask and made up to mark with distilled water. The solutions were filtered and stored in plastic bottles for the analysis of Na, K, Ca, Mg, Cu, Zn, Pb and Cd using Atomic Absorption Spectrophotometer (AAS).

Phytochemical Determination
10 g each of the powered plant leaves and stem bark were soaked in ethanol for 24 hours. The extracts were filtered through a Whatman filter paper. The filtrates were then concentrated using a rotary evaporator with the water bath set at 40^0C [15].

The phytochemical analyses of the extracts were carried out according to the methods described by Trease and Evans [18] as follows:

Statistical Analysis
Data generated was subjected to Analysis Of Variance (ANOVA) to determine the level of significance using statistical software (CROPSTAT 7.2.3 International Rice Research Institute IRRI, Philippine). Significant means were separated using least significant difference (LSD) technique as described by Gomez and Gomez, [19]. Differences were considered significant if probability is less than 5% ($p \leq 0.05$) for all the data.

RESULTS AND DISCUSION

Phytochemical Screening
Table1 presents the results of phytochemicals obtained in the leaves and stem bark of *Haematostaphis barteri* from 10 locations in Hong local Government area. The leaves and the stem bark demonstrated the presence of tannin in all the samples screened. The leaves showed the presence of alkaloid in all the samples from the locations studied. Glycosides were not observed in the entire samples investigated. The parts from Uba area showed the presence of flavonoid. The stem bark contained flavonoid in all the samples screened. Saponin was also recorded in all the parts analyzed.

Table 1: Phytochemical screening *of Haematostaphis barteri* extracts

Location	Plant parts	Tannin	Alkaloid	Glycoside	Flavonoid	Saponin
Pella	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	+
Hong	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	+
Hildi	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	+
Uba	Leaves	+	+	-	+	+
	Stem bark	+	-	-	-	+
Gaya	Leaves	+	+	-	+	+
	Stem bark	+	-	-	-	+
Fa'a	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	+
Garaha	Leaves	+	+	-	-	+
	Stem bark	+	+	-	+	+
Gashala	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	+
Mugili	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	+
Pubba	Leaves	+	+	-	-	+
	Stem bark	+	-	-	+	-

= present, - = absent

Elemental determination

Table 2 shows the mean distribution of essential elements in *Haematostaphis barteri* leaves among ten locations in Hong Local Government Area. Na was recorded highest (4.100µg/g) around Hong area. The amount for K varies from 0.070µg/g to 0.900µg/g with an overall average of 0.083µg/g. The values for Mg however, ranged between 0.230 to 0.300µg/g. Ca was also recorded in the entire samples with highest value around Gashala (0.253µg/g). No significant difference was observed with regards to Na, K, Mg and Ca contents among the locations studied at $p \leq 0.05$. The results also indicate the presence of Cu in all the locations with highest value around Uba (0.430µg/g) area. For Zn content, the values ranged from 0.400 to 0.520µg/g among the locations investigated. The amount for Zn was below the detectable limit (<0.06µg/g) of the instrument used. Cd was recorded in all the samples with an overall mean of 0.280µg/g. there was no significant difference ($p \leq 0.05$) among the locations in terms of Cu and Zn contents, but significant difference was observed in Cd.

Mean distribution of elements in *Haematostaphis barteri* stem bark among ten locations in Hong Local Government Area is presented in Table 3. The results showed that Na content varies from 0.760µg/g and 0.810µg/g. the value for K was highest in the sample from Gashala area (0.440µg/g). The amount for Ca ranged between 0.490 to 0.600µg/g with a total average value of 0.517µg/g. no significant difference was observed with regard to Na, K, Mg and Ca among the locations at $p \leq 0.05$. The results indicate that Cu was present in all the samples from the locations studied with a total mean value of 0.390µg/g. the value for Zn was only recorded in sample from Pella (0.038µg/g). Pb content was below the detectable limit (0.06µg/g) of the instrument used. Cd compositions among the locations ranged between 0.150µg/g to 0.370µg/g, but there was no significant difference ($p \leq 0.05$) observed among the locations in terms og Cu, Zn and Cd contents.

Table 2: Mean levels of Elements in *Haematostaphis barteri* leaves (µg/g dry weight)

	Elements							
Location	Na	K	Mg	Ca	Cu	Zn	Pb	Cd
Pella	4.00	0.070	0.260	0.100	0.410	0.406	<0.060	0.310
Hong	4.100	0.090	0.250	0.100	0.400	0.413	<0.060	0.300
Hildi	3.900	0.080	0.240	0.100	0.420	0.410	<0.060	0.300
Uba	4.000	0.080	0.260	0.110	0.430	0.400	<0.060	0.310
Gaya	3.980	0.090	0.230	0.110	0.400	0.400	<0.060	0.280
Fa'a	4.000	0.900	0.240	0.090	0.410	0.430	<0.060	0.300
Garaha	4.000	0.800	0.250	0.080	0.400	0.420	<0.060	0.290
Gashala	3.800	0.800	0.230	0.253	0.400	0.410	<0.060	0.290
Mugili	3.990	0.700	0.250	0.100	0.340	0.310	<0.060	0.180
Pubba	3.8	0.100	0.3	0.090	0.310	0.520	<0.060	0.240
Mean	3.9	0.083	0.251	0.113	0.390	0.412	0.258	0.280
SE	0.534	0.011	0.019	0.042	0.031	0.017	0.626	0.021
LSD(5%)	1.586	0.033	0.057	0.125	0.092	0.049	0.001	0.063

Any two mean having difference greater than LSD are significantly different at 5% level of probability.

Table 3: Mean levels of Elements in *Haematostaphis barteri* Stem bark (µg/g dry weight)

	Elements							
Location	Na	K	Mg	Ca	Cu	Zn	Pb	Cd
Pella	0.780	0.400	0.840	0.500	<0.025	0.038	<0.060	0.370
Hong	0.800	0.420	0.820	0.530	<0.025	<0.008	<0.060	0.160
Hildi	0.793	0.430	0.840	0.520	0.145	<0.008	<0.060	0.180
Uba	0.790	0.420	0.820	0.490	0.115	<0.008	<0.060	0.170
Gaya	0.780	0.410	0.830	0.500	<0.025	<0.008	<0.060	0.180
Fa'a	0.770	0.430	0.840	0.510	<0.025	<0.008	<0.060	0.160
Garaha	0.790	0.420	0.830	0.500	<0.025	<0.008	<0.060	0.150
Gashala	0.760	0.440	0.830	0.520	<0.025	<0.008	<0.060	0.190
Mugili	0.790	0.420	0.800	0.500	0.220	<0.008	<0.060	0.160
Pubba	0.810	0.330	0.940	0.600	0.100	<0.008	<0.060	0.200
Mean	3.158	0.410	0.839	0.517	0.072	0.019	0.060	0.192
SE	0.341	0.0320	0.026	0.041	0.047	0.010	0.000	0.060
LSD(5%)	0.223	0.097	0.079	0.012	0.014	0.030	0.002	0.180

Any two mean having difference greater than LSD are significantly different at 5% level of probability.

Phytochemical Compositions

Tannin: Tannins were observed in the entire samples screened. Tannins are widely distributed in many plants species. They have astringent properties which are important anti-oxidant and in wound healing [20].

Alkaloid: The leaves demonstrated the presence of alkaloids in all the samples from the 10 locations investigated. Alkaloids are produced by large variety of organisms, plants and animals. They almost uniformly invoke bitter taste [21]. They have pharmacological effects and often used as medications and recreational drugs.

Flavonoid: The stem bark showed the presence of alkaloids in the samples screened, except from Uba which demonstrated in both the leaves and the stem bark. Kubmarawa *et al.*, [22] reported flavonoid in the aerial parts. Flavonoids are the most common group of polyphenolic compounds in the human diet and are found in plants [23]. The widespread distribution of flavonoids and their low toxicity compared to other active plant compounds means that many animals, including humans, ingest significance quantities in their diet. Flavonoids have anti-allergic, anti-inflammatory, anti-bacterial, anti-cancer, anti-diarrhea and anti-oxidant properties [24,25].

Glycoside: Glycosides were not recorded in the entire samples investigated by the methods used. Glycosides play important roles in living organisms. They are used as medications for treatment of congestive heart failure and cardiac arrhymia [26].

Saponin: The entire samples showed the presence saponins. Saponins have the potential to lower cholesterol levels in humans due to their hypocholesterlemetric effect. They form complexes with cholesterol to reduce plasma cholesterol [9].

Elemental compositions

Sodium: Considerable amount of sodium was recorded in the leaves and stem bark of the samples investigated with highest amount in the leaves around Hong area (4.100µg/g). Sodium is one of the chief extracellular ions in the body. It involves in the production of energy, transport of amino acids and glucose into the body cells and its deficiency results in hyponatremia [29].

Potassium: The overall mean values for potassium in the leaves and stem bark were 3.900µg/g and 3.158µg/g respectively. The amounts of potassium in the samples investigated are within the safety limits of 10 to 100µg/g recommended by WHO [30]. Potassium is the principal intracellular cation. It helps to regulate osmotic pressure and pH equilibrium. The recommended daily intake is 4700 mg [31]. Its deficiency causes muscles weakness, decrease reflex responses and respiratory paralysis.

Magnesium: The mean values for magnesium recorded in the leaves and stem bark of the samples were 0.251µg/g and 0.839µg/g respectively, which was lower than the amounts obtained by Kubmarawa *et al*., [22]. Magnesium plays important role in maintaining electrical potential in nerves and membranes. It improves insulin sensitivity, protect against diabetes and its complications and also reduce blood pressure [33].

Calcium: The leaves and stem bark contained a total mean value of 0.113µg/g and 0.517µg/g respectively which were comparatively lower than the results (1.73µg/g) reported by Kubmarawa *et al*., [22]. The results obtained are within the range of permissible limit as prescribed by WHO [30]. Calcium is needed for muscles development, heart and digestive System. It is also essential for the normal development and maintenance of bones [31].

Copper: The leaves recorded presence of copper in all the samples from the 10 locations investigated with an overall mean value of 0.390µg/g. The stem bark from Hildi, Uba, Mugili and Pubba areas showed the presence of copper only with a mean value of 0.072µg/g. Copper plays important role in treatment of chest wound and prevent inflammation arthritis and similar diseases. It is also essential for the formation haemoglobin of the red blood cells. It is required by trace quantity by humans [33].

Zinc: The values for zinc in the stem bark were below the detectable limit (0.008µg/g) of the instrument used with exception of the samples from Pella area. The leaves contained measureable amounts in all the samples with a mean value of 0.412µg/g which was lower than 6.00µg/g observed by Kubmarawa *et al*., [3]. Zinc is essential constituents of enzymes involve in carbohydrate and protein metabolism and nucleic acid synthesis. Its deficiency results in impaired growth and development, skin lesion and loss of appetite [31].

Cadmium: Cadmium was recorded in the entire samples with average concentration of 0.280µg/g and 0.192µg/g in the leaves and stem bark respectively. The amount recorded for Cadmium was slightly above the 0.20µg/g fresh weight recommended as the maximum permissible level of cadmium in vegetables as reported by FAO/WHO [34], but lower than the results reported in amaranthus (15.83µg/g) by Shagal *et al*., [10] and in kenaf (2.04µg/g) and roselle (2.07µg/g) by Yahaya *et al*., [34].

Lead: Lead was below the detectable limit (0.060µg/g) of the instrument used. Lead has low geochemical mobility and bioavailability. Its transportation to aboveground tissues in plants is minimal due to its retention in roots and precipitation [35]. An ultra structural study using transmission electron spectroscopy revealed the retention of lead in the cell wall of roots, particularly around intercellular spaces [36]. This explains why lead was not observed in the leaves and stem bark of the plants investigated by the analytical instrument used. Lead is toxic metal and non-essential element for human body as it causes a rise in blood pressure, kidney damage and miscarriage [32].

CONCLUSION

The results reported in this study confirm that the leaves and stem bark plant investigated contained tannins, saponins, alkaloids and flavonoids. Considerable amount of some essential elements (Na, K, Mg and Ca) and heavy metals (Cu, Zn and Cd) were also recorded within safe limits as prescribed by WHO [30], except for cadmium whose concentration was slightly above the safety limit (0.20µg/g). However, the results for cadmium contents in the samples investigated were lower than the results reported in cabbage (41-55µg/g) and amaranths (6.oµg/g) by Mohammed and Khamis, [37]. This is an important result as human health is directly affected ingestion of plant parts. Biomonitoring of essential and trace elements in the leaves and stem bark of plants need to be investigated because these are the main source of food and medicine for humans in most of the African countries.

REFERENCES

[1] Bokhari, M.H. and Ahmed, M.J. **(1979)**. Food Plants in Borno State Nigeria. University Press, Maiduguri, Nigeria. Pp. 20 – 21.
[2] Barminas, J.T. Charles and Emanuel, D. **(1998).** *Foods in Human Nutrition*, 53: 29 – 38.
[3] Kubmarawa, D. Andenyang, I. F.H., Magomya, A.M. **(2008)**. *African Journal of Biotechnology,* 7(18): 888 – 889.
[4] Humphery, C.M., Clegg, M.S., Keen, C.I. and Grivetti, L.E. **(1993).** *Int. J. Food Sci. And Nutr.*, 44: 1-6.
[5] Freiberger, D.J., Vanderjagt, A., Pastuszyn,R,S., Glew, G., Mounkaila, M. and Glew, R.H. **(1998)**. *Plant foods for Human Nutrition*. 53: 57-69.
[6] Adenipekun, C.O. and Oyetunji, O.J. **(2010).** *Journal of Applied Biosciences* 35: 2294 – 2300.
[7] Steinmetz, K.A. and Potter, J.D. **(1996)**. *Journal of American Diet Association* 10: 1027 – 1030.
[8] Starvic, B. **(1994).** *Journal of food Chemistry and Toxicology*. 32: 79-90.
[9] Nnam, N.M., Onyechi, J.C. and Madukwe, E.A. **(2012).** *Nigerian Journal of Nutritional Science.* 33(2), 15-19.
[10] Shagal, M.H; Maina, H.M; Donatu, R.B and Tadzabia, K. **(2012).** *Global Advance Research Journal of environmental science and Toxicology*. 20: 018-022.
[11] Sharma, R.K., Angrawal, M. and Marshal, F.M. **(2007).** Ecotoxicology and Environmental Safety. 66: 258 – 266.
[12] Nwajei, G.E. **(2009).** *American-Eurasian Journal of Sustainable Agriculture.* 3: 574-578.
[13] Ladeji, O. Ahin, C.U. and Umaru, H.A. **(2004).** *Africa Journal National Science* 7: 71 – 73.
[14] Nkafamiya, I.I. Osemeahon, S.A, Modibo, U.U. and Aminu, A. **(2010).** *African Journal of food Science* 4; 104 – 108.
[15] Ayoola, G.A., Coker, H.A.B., Adesegun, S.A., Adepoju-Bello, A.A., Obaweya, K., Exennia, E.C. and Atangbayila, T.O. **(2008).** *Tropical Journal of Pharmaceuticals Research* 7(3): 1019 – 1024.
[16] Crosby, T.N and Patel, I. **(1995).** "General Principles of Good Sampling Practices" The Royal Society of Chemistry. Teddington. Pp. 1 – 17.
[17] Onwuka, G.I. **(2005).** Food Analysis and Instrumentation Theory and Practice. Naphatali Prints, Nigeria. Pp. 126 – 129.
[18] Trease, G.E. and Evans, W.C. **(1989).** Pharmacognosy. 3rd edition. Bailliere Tindall, London. Pp. 176 – 180.
[19] Gomez, A.K. and Gomez, A.A. **(1984).** Statistical procedure for Agricultural Research. International Rice Research Institute, John Wiley and Sons Inc., New York. Pp. 680.
[20] Mustapha, A.J; Fanna, I.A; Irfan, Z.K and Umar, K.S. **(2012).** *International Journal of Basic and Applied Chemical Sciences* 1: 99-106.
[21] Rhoades, D.F. **(1979).** Evolution of Plant Chemical Defence against Herbivores. New York. Academic Press. Pp. 41.

[22] Kubmarawa, D; Ajoku, G.A; Enwerem, N.M and Okorie, D.A. **(2007).** *African Journal of Biotechnology.* 6(14): 1690-1696.
[23] Spencer, P.E. **(2008).** *British Journal of Nutrition.* 99: 60 – 77.
[24] Schuler, M. Sies, H. Illek, B. and Fischer, H. **(2005).** *Journal of Nutrition.* 135(10); 3320-5.
[25] Cushine, T.P.T. and Lamb, A.J. **(2011).** *International Journal of Antimicrobial Agents.* 38(2): 99 – 107.
[26] Brian, F.H; Thomas-Bigger, J and Goodman, G. **(1985).** The Pharmacological Basis of Theuraupeutic, Macmillan, 7th edition New York, NY, USA. Pp. 123-124.
[27] Donatelle, R.J. **(2005).** Health: The Basics. 6th edition, Pearson Education Publishers. San Francisco, Pp. 20.
[28] World Health Organization **(1996).** Trace elements in Human. Health and Nutrition. Geneva, Switzerland, WHO Publications.
[29] Norman, N. and Joseph, H.H. **(1996).** Food Science, Chapman and Hall New York. 5th edition Pp. 55 – 62.
[30] Kiran, Y.K., Mir, A.K., Rabia, N., Mamoona, M., Hina, F., Nighat, S., Tasmia, B. and Ammarah, K. **(2011).** *African Journal of Pharmacy and Pharmacology.* 5(3): 317 – 321.
[31] Afshin, A. And Masoud, A.Z. **(2008).** *Asian J. Trop. Med. Public Health.* 39(2): 335-340
[32] Yahaya, Y; Birnin-Yauri, U.A; Bagudo, B.U and Noma, S.S. **(2012).** *Journal of Soil Science and Environmental Management.* 3(8): 207-215.
[33] Brenna, M.A. and Shelly, M.L. **(1999).** *Ecol. Eng*., 12: 271-297.
[34] Wenger, K., Gupta, S.K.,Furrer,G. and Schulin,R. **(2003).** *J. Enviro. Qual.* 32: 1669-1676.
[35] Mohammed, N.K and Khamis, F.O. **(2012).** *Natural Science.* 4(8): 588-604.