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PHYTOCHEMICAL, ANTIMIROBIAL SCREENING AND HUMAN HEALTH RISK ASSESSMENT OF HEAVY METALS OF STEM-BARK AND ROOT EXTRACTS OF NEWBOULDIA LAEVIS (BOUNDARY TREE)

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ABSTRACT

Increasing awareness of multi-drug resistant strains of microorganisms and the hazards associated with the use of synthetic / orthodox drugs has increased investigations on plant extracts as possible alternative drugs. Extracts of Newbouldia laevis stem-bark and root were subjected to phytochemical and antimicrobial screening as well as heavy metal concentration content. The aim was to investigate the potentiality of using the extracts as alternative to synthetic / orthodox drugs and conduct ecological, and human health risk assessment of heavy metals in the extracts. Phytochemical screening of the extracts was carried out using standard methods. Antimicrobial activities of the aqueous, methanol and n-hexane extracts were carried out using agar well diffusion method. The test organisms were laboratory isolates of Salmolella tyhpi, Staphylococcus aureus, Escherichia coli, Pseudomanas aeruginosa, Aspergellus flavus, Aspergillus niger, and Candida albican. Metal concentration of the extracts was carried out using Atomic Absorption Spectrometry (AAS). Phytochemical screening showed the presence of the following phytochemicals in the stem-back and root extracts: alkaloid (1.00%, 4.00%), flavonoids (7.00%, 0.60%), saponins (10.00%, 11.00%), cardiac glycosides (2.24%, 3.06%), steroids (2.50%,4.87%), terpenoids (1.80%, 1.26%), and tannins (6.00%,10.00%) respectively. The survey of heavy metal concentration was Zn (12.10 mg/kg), Mn (3.45 mg/kg), Cr (4.55) and Zn (10.80 mg/kg), Mn (2.88 mg/kg) and Cr (4.99 mg/kg) in stem bark and root extracts respectively. Cd, Cu and Pb were not detected in both extracts. This showed that the levels of concentrations of the metals determined were generally below the maximum permissible limits established by International regulatory bodies. All the extracts inhibited growth of the test organisms. The aqueous and ethanol extracts proved more potent than the positive control (tetracycline). The results of ecological risk assessment showed PERI, Cdeg, mCdeg and PLI values of 0.8097, 0.1423, 0.0474 and 0.1222 respectively for the stem bark extract and 0.7015, 0.1332, 0.0444 and 0.1139 respectively for the root extract. Human health risk assessment of the heavy metals gave a total hazard index of 0.0764 and 0.0667 for stem bark and root extracts respectively. These ecological and human health risk assessment results indicated no risk in taking the extracts as alternative medicine. This study therefore has justified ethno-medical use of the plant for the treatment of diseases caused by these pathogens.

Keywords: Newbouldia laevis, Phytochemical, Antimicrobial, Extracts, Haman health, Risk assessment.

INTRODUCTION

The use of traditional medicine dates back to the beginning of mankind in Africa, as man in his quest to achieve good and healthy living examines all aspect of his environments by trial and error [1, 2]. Over 90% of the World's population still rely on plant species for food, medicine, fodder and wood uses [3, 4]. Medicinal plants represent a rich source of antimicrobial agents. They are used medicinally in different countries and are source of many potent and powerful drugs.

Every community in Nigeria has peculiar herbs and plants which are used in some ways for the treatment of symptoms and diseases vary from skin rash to

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cancer [5, 6]. Plants contain active components (secondary metabolites) which possess medicinal properties that are harnessed for the treatment of different diseases. Screening of plant drugs will continue for the development of new pharmaceuticals to resolve both old and new health challenges.

Newbouldia laevis plant, native of tropical Africa, grows from Guinea savannah to dense forest. It is an African folk medicine for the treatment of malaria, stomach ache, tooth ache, breast cancer, diarrhea, pile, epilepsy inflammation and others [7, 8]. The decoction of the pounded roots has been reported to be used in the treatment of intestinal problems and syphilis [9]. A macerated or decoction of the root is taken by mouth as a vermifuge to rid the body of round worm, and is also used to treat hernia and syphilis. Root scraping, combined with chilli pepper are put into a carious tooth [10].

Studies abound in the application of Newbouldia laevis plant in ethno-medicine [11-16], building and construction, fire retardancy [17-19] and a host of other applications but there is little or no document on ecological and human health risk assessment of potential toxic metals which the plants absorb from soils as they absorb mineral nutrients from the soil for healthy living.

The goal of this study is to look into phytochemicals and antimicrobials in the stem-bark and root extracts of Newbouldia laevis in order to determine their inhibitory potency in some pathogenic organisms and to look into their overall therapeutic use, as well as to assess the ecological and human health risks of potential toxic metals.

MATERIALS AND METHODS

Plant collection, identification, and preparation

The stem-bark and root of Newbouldia laevis were harvested from the herbarium of Chukwuemeka Odumegwu Ojukwu University, Anambra State, Nigeria. It was identified by a taxonomist, Prof J.C. Okafor of No 7, Donna drive, Ogui, Enugu, Enugu State. The plant parts were cut into small sizes, washed and kept under mild sun for two days before transferring it under shade for two weeks. They were thereafter pulverized using manual grinder. The pulverized samples were stored in transparent plastic cans with screw covers until needed for analysis.

Extraction

The pulverized stem-bark sample (10 g) was placed in a beaker and macerated for 24 hours in 200 mL of ethanol. Extensive extraction was achieved by repeating the extraction process. Filtration was carried out with Whatman No. 1 filter paper, and the filtrate was concentrated to one third (1/3) of its original volume in a water bath at 40 oC. This was done on the root sample as well.

Phytochemical Screening

The crude extracts were evaluated for the presence of alkaloids, flavonoids, tannins, saponnins, terpenoids, and steroids using standard methods [20–22].

Heavy Mineral and Toxic Metal Analysis

Sample digestion and metal determination About 2 g each of the crude extracts of N. laevis wet-digested with 10 mL of HNO3. The digested samples were cooled, filtered, and transferred to 25-mL standard flasks. The flasks were made to mark with deionized water, and the metal concentration were determined with an Atomic Absorption spectrophotometer (Varian AA240, United States) equipped with air-acetylene fame.

Antimicrobial assay

The antimicrobial activities of crude aqueous, ethanol, and n-hexane extracts were determined using the agar diffusion method as described by Barry and Thorsberry, 1985 [23]; and Bauer et al 1966 [24]. Nutrient agar was used as growth media for the microbes. Nutrient agar medium was prepared by dissolving 7g of agar in 250mL of distilled water in a flask. It was sterilized in an autoclave at 121oC for 15minutes, cooled and transferred into petri-dishes for solidification. Cultures of Salmonella typhi,

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Staphylococcus aureus, Escherichia coli, pseudomonas aeruginosa, Aspergellus flavus, Aspegellus niger, and Candida albican were respectively grown on nutrient and sabouraud dextrose agar using sterile cotton swabs. Wells of 4 mm in diameter were cut from the agar plate using a cork borer. A sterile micropipette was used to introduce 1 mL of the crude aqueous extract containing 0.1 mg/mL into the well. The same petri dish setup was used each time, with 0.05 mg/mL tetracycline as a negative control and also 0.05 mg/mL amphotericin B (a positive control for fungi). The petri-dishes were incubated with the base upwards at 35 oC for 24 hours for bacteria and at 25 oC for 48 hours for fungi. After the incubation, the plates were examined for inhibition zones and the observed zones were measured and recorded in millimeters. This same procedure was repeated with ethanol and n-hexane crude extracts

Ecological risk assessment

Four ecological indices (potential ecological risk index PERI, degree of contamination Cdeg, modified degree of contamination mCdeg, pollution load index (PLI) were assessed for potential toxic metals detected in the extracts. PERI was conducted to evaluate the ecological risk index

(ERI) using the version by Hakanson (1980) [25]. The PERI is represented in Eq. 1:

$$PERI = \sum(Cf \times Trf) \quad (1)$$

where Trf is the toxic response factor (Cd=30, Cr=2, Pb=5, Cu=5, Zn=1, and Mn=1), Cfis the contamination factor (mean metal concentration (C)divided by the pre-industrial concentration, Co (mg/kg). Co for Cr, Zn, and Mn is 100, 421, and 5 respectively.

$$Cf = C/ Co \quad (2)$$

The risk grade indices and grades of potential ecological risk of potential toxic metal pollution as provided by Hakanson (1980) [25] are presented in Table 1. The degree of contamination, Cdeg estimates the degree of overall contamination in the extracts is calculated from (Eq. 3). A modified model for calculating the prevailing degree of contamination as proposed by Abraham and Parker (2008) is given in Eq. 4. where n is the number of metals assessed and mCdeg is the modified degree of contamination. The modification has an edge by giving room for as many metals as possible in contamination determination. The pollution load index (PLI) permits the comparison of pollution loads of different extracts. The PLI was determined using Eq. 5 [26].

$$\text{Cdeg} = \sum_{i=1}^{n} Cf \quad (3)$$

$$\text{mCdeg} = ((\sum_{i=1}^{n} Cf)/\text{n})\ \text{Cf} \quad (4)$$

$$\text{PLI} = \sqrt[n]{Cf1xCf2xCf3 \ldots\ldots xCfn} \quad (5)$$

Table 1: PERI grades

PERI	Potential ecological risk grade
< 40	Low
40 to 79	Moderate
80 to 159	Considerable
160 to 319	High potential
≥ 320	Significantly high

Source: Hakanson (1980) [25]

Human Health risk assessment

Carcinogenic and non-carcinogenic health risk assessment methods are usually used to determine potential health risks of pollutants. The probability of cancer risk is used to determine the health risk of carcinogenic pollutants, while target hazard quotient (THQ) is used to estimate the non-carcinogenic risks [27]. Metals find their way into the human body through ingestion, dermal and inhalation. The current work considers injection only since the extracts are to

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taken by ingestion as alternative to orthodox medicine. The non-carcinogenic health risk resulting from ingestion of the extracts was evaluated using THQ and total hazard index (THI) [28-30] as adopted by Ihedioha et al., 2021 [31]. THQ is the ratio of estimated intake of a pollutant to the oral reference dose (RfD) while THI evaluates the perceived risk from a mixture of chemical contaminants. When the THQ is less than unity, it is assumed that there will be no probable health risk but when it is greater than unity, it shows concern on the health exposure to pollutants [32].THQ method of assessment provides an indication of the health risk status owing to these pollutants This method has been used and had proven to be factual and worthwhile [33]. In determining THI, we first obtain EDI and THQ as employed by Ibe et al., 2021 [34] and Enyoh and Isiuku, 2021 [35].

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical screening of the Newbouldia laevis revealed the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, steroids, and cardiac glycosides and their amounts as shown in Table 2. Alkaloids are used therapeutically as the most efficient plant substance. They exhibit variety of biochemical, psychopharmacological and behavioral effects in animals and humans [36].

The presence of flavonoids in the root of Newbouldia laevis supports its use in the treatment of diabetes and heart disease [37]. Flavonoids exhibit wide range of biological activities in which either to scavenge for hydroxyl radicals or superoxides anions radicals and thereby exhibiting health promoting activities.

Cardiac glycoside in the root of Newbouldia laevis support its use in the treatment of congestive heart failure [38]. Presence of tannins strongly supports its use for the healing of hemorrhoids, frost bite, and varicose ulcer [39, 40]. The antioxidant activity of tannins results from their free radical and reactive oxygen species-scavenging properties as well as the chelation of transition metal ions that modify the oxidation

Table 2: Results of Phytochemical analysis of N. laevis

Phytochemical	Quantity (%)		Quality	
	Stem-bark	Root	Stem-bark	Root
Alkaloids	1.00	1.00	+	+
Flavonoids	7.00	7.00	++	++
Tannins	6.00	6.00	++	++
Saponins	10.00	10.00	+++	+++
Terpenoids	1.08	1.08	+	+
Steroids	2.53	2.53	+	+
Cardiac glycosides	2.24	2.24	+	+

Key: + = sparingly present, ++ = moderately present, +++ = highly present

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Mineral and Toxic Metal content

From Table 3, the minerals present in the seed were within the World health organization (WHO) recommended permissible limits.

Table 3: Results of Mineral and Toxic Metal content of N. laevis.

Element	Concentration (ppm)		
	Stem-bark	Root	WHO*
Potassium	2.23	0.28	10-100
Zinc	12.10	10.80	27.4
Sodium	1.22	0.36	400-500
Magnesium	5.45	2.88	2000
Calcium	0.54	0.18	3600-80000
Copper	Nd	Nd	100-300
Iron	0.69	0.27	50-500
Chromium	4.55	4.99	-
Mercury	Nd	Nd	0.001
Lead	Nd	Nd	-
Cobalt	Nd	Nd	0.01
Manganese	3.45	2.88	-
Cadmium	Nd	Nd	.

Nd = Not detected, * Source [41]

They play important roles from physiochemical, technological, and nutritional point of view. Potassium as an electrolyte is vital to the healthy functioning of all the body cells tissues, and organs. It also helps to control the amount of water in the body and maintain a healthy blood pH level. Potassium is particularly important for the ability of the skeletal and smooth muscles to contract, so important for regular digestive and muscular functioning. Zinc is so important in human body because in every tissue in the body. It is directly involved in cell division, powerful antioxidant, helping to prevent cancer, and directly involved in maintenance of ideal hormone level [42, 43]. Sodium plays in nerve function and muscle contraction. Calcium is responsible for the building of bones and teeth and regulating certain body processes. Absence of toxic metals is a pointer that the plant root can be taken without fear of metal poisoning.

Antimicrobial activities

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The result of the antimicrobial activities on the seven pathogenic microorganisms as presented in Table 4 showed that both aqueous, ethanol, and n-hexane stem-bark and root extracts were active against both gram positive and gram-negative bacteria and fungi.

The antimicrobial activity shown by these extracts is due to phytochemicals present in the plant. Aqueous stem extract showed the highest activity against E. coli with inhibition zone of 12.48mm and least activity against A. flavus with inhibition zone of 4.00mm whereas the aqueous root extract showed highest activity against E. coli (16.50mm) and least activity against A. flavus. Ethanol stem-bark extract had the highest activity against E. coli (14.22) and least activity against A. niger (2.00mm), whereas the ethanol root extract had the highest activity against S. aureus with inhibition zone of 14.00mm and least against P. earuginosa with inhibition zone of 2.0mm. The n-hexane stem-bark extract had the highest activity against A. flavus (13.84mm) and least activity against C. albican (2.22mm) while the root extract had highest activity against P. aeruginosa and least activity against S. typhi with inhibition zone of 13.36mm and 1.52mm respectively

Table 4: Results of Antimicrobial activities of N. laevis

Tested organism	Zone of Inhibition (mm)							
	Water extract		Ethanol extract		n-Hexane extract		+ve control (Tetracycline)	-ve Control (Water)
Salmonella typhi	11.00	4.88	8.62	12.00	5.34	1.52	22.00	NA
S.aureus	8.50	12.62	11.86	14.00	2.86	5.32	26.18	NA
E.coli	12.48	16.50	14.22	10.80	4.00	8.16	19.66	NA
Psedomonas aeroginosa	10.00	6.40	4.70	2.00	7.62	13.36	21.00	NA
Aspergillus flavus	4.00	4.18	9.20	7.20	13.84	11.29	18.30	NA
Asperrgillus niger	7.52	5.44	2.00	8.60	6.32	6.62	21.7	NA
Candida albican	12.60	12.58	10.02	9.12	2.22	3.32	5.52	NA

NA = No action; Concentration of extract = 0.5mg/ml

The extracts exhibited considerable inhibition activities against all the test organisms. Some extracts relatively have comparable inhibition activities to the reference drugs used as control. The discrepancy in inhibition zones diameter for the test organisms could be attributed to difference in polarity of solvents used and possible phytocompounds they could extract as well as their ability to dissolve or diffuse in the media used in the assay [44].

Ecological risk assessment

The risk indices of the heavy metals in the extracts are presented in Table 5. It showed that all the metals fell within low-risk category (< 40). This suggests that these pollutants pose no ecological risk

The extracts have low contamination factor in all the metals, all having a contamination factor < 1. The results of mCdeg for the extracts are stem bark

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(0.0474) and root (0.0444) indicating a very low degree of contamination. Abrahim & Parker, 2008 in their work used. It is established that mCdeg < 1.5 (very low degree of contamination), 1.5 ≤ mCdeg < 2 (low degree of contamination), 2 ≤ mCdeg < 4 (moderate degree of contamination), 4 ≤ mCdeg < 8 (high degree of contamination),8 ≤ mCdeg < 16 (very high degree of contamination), 16 ≤ mCdeg < 32 (extremely high degree of contamination) and > 32 (ultra-high degree of contamination) [31, 45, 46].The PLI results (0.1222) for the stem bark extract and (0.1139) for the root extract showed very low pollution load

Table 5: Risk indices of potential toxic metals in the extracts

Metal	Zn	Mn	Cr	PERI	Cdeg	mCdeg	PLI
	Concentration	(mg/Kg)					
Stem bark	12.10	3.450	4.550	0.8097	0.1423	0.0474	0.1222
Cf	0.0287	0.6900	0.0455				
Cf x Fr	0.0287	0.6900	0.0910				
Root	10.80	2.88	4.99	0.7015	0.1332	0.0444	0.1139
Cf	0.0257	0.5760	0.0499				
Cf x Fr	0.0257	0.5760	0.0998				

Health risk exposure assessment

The estimated daily intakes of Zn, Mn and Cr were presented in Table 6. The daily intakes for a 60-kg adult were compared with tolerable daily intakes as stipulated by WHO, JECFA and NRC. The daily intakes of Zn (0.1350), Mn (0.00386) and Cr (0.0051) for the stem bark and Zn (0.120), Mn (0.0032) and Cr (0.0056) for the root were very low, and comparable to safe values. Low daily intake has also been reported by Zhuang et al. (2009) [47] and Ihedioha et al. (2016) [28] on rice grains. The EDI of the potential toxic metals through the ingestion of the extracts used in ethno-medicine followed thus: Zn > Cr > Mn for the stem bark and the root extracts respectively. The HQ trend also follows the same trend. The total hazard index (THI) was less than 1 (0.0764) and (0.0667) for the stem bark and the root extracts respectively indicating no probable health risk.

Table 6: Exposure daily index, target hazard quotient and total hazard index

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Metal	Zn	Mn	Cr	
		Stem bark		
EDI	0.01350	0.00386	0.0051	
THQ	0.045	0.028	0.0034	
THI				0.0764
		Root		
EDI	0.01200	0.0032	0.0056	
THQ	0.040	0.023	0.0037	
THI				0.0667

CONCLUSION

The extracts of the stem-bark and root of Newbouldia laevis plant has shown to be potent medicinal plant for pharmaceutical treatment of diseases caused by the selected tested organisms without posing ecological and human health risks. Therefore, in order to check the trend of increased emerging and resultant infectious diseases, a multi prolong approach that involves the development of new drugs is required. The results obtained in this research may provide support to the use of plant in traditional medicine.

REFERENCES

1. J. M. Dalziel (1961), The Useful Plants of West Tropical Africa, the Crown Agent, London.

2. E. A. Sofowora (1983), Medicinal Plants and Traditional Medicine in Africa, 2nd edn. John Wiley,Winchester.

3. A. Nfotabong-Atheull, N. Din, I. G. Essome-koun, B. Satyanarayaus, U. Kaedam, F. Dahdouh-Guebas (2011). Assessing forest

4. products usage and local resistant: perspectives of environmental changes in semi-urban and rural mangrove of Cameroon, Central Africa, *J. Ethnobiol Ethnomed* **7**,14.7-41

5. F. G. Vodouhe, O. Coulibaly, C. Greane, B. Sinsin (2009), Estimating the local value of non-timber forest products to Pendijari biosphere reserve dwellers in Benin. *Eco.Bot.*, **63**, 397-412

6. H. O. Edeoga, D. E. Okwu, and O. Mbaebie (2005), Nutritional values of some known conventional leafy vegetables of Nigeria, *African Journal of Biotechnol*, **4(7),** 685-688.

7. J. Filogona, C. S. Dunah and P.S. Walkama (2005). An invitro study of the antimicrobial activity of the root extract of *Calotropis procena* and *Moringa oleifera. Ife Journal of Science*,

ISSN 0795 - 22066

7(1),43-44.

8. M. M. Iwu (1983), Traditional Igbo Medicine. Institute of African Studies, University of Nigeria Nsukka, 122-144.

9. D. N. Akunyili (2000), Anticonvulsant activity of the ethanol extract of *Newbouldia laevis*. 2nd NAAP Scientific Conference,1555-158.
10. Tropical Plkants Database, Ken Fern. Tropical. Theferns.info. retrieved 05 07 2016.

11. J. B. Harborne (1984), Phytochemical methods, A guide to modern techniques of plant analysis. 2nd Ed. Chapman and hall, New York, 196-197.

12. H. Usman and J. C. Osuji, (2007). Phytochemical and in vitro antimicrobial assay of the leaf extract of *newbouldia laevis*, *Afr. J. Trad. CAM*, **4(4)**, 476 – 480 476

13. A. J. Aladesanmi, R. Nia, and A. Nahrstedt (1998), New Pyrazole alkaloids from the root bark of *Newbouldia leavis*, *Planta Medica.* **64**, 90 – 91.

14. H. Usman, A. H. Yaro and M.M. Garba (2008), Analgestic and Anti-inflammatory screening of *Newbouldia leavis* flower in rodents, *Trends in Medical Research*, **3(1),** 10-15.

15. S. Gafner, J. L. Wolfender, M. Nianga and K. Hostettmann (1997), Phenylpropanoid glycosides from *Newbouldia laevis* roots. *Phytochemistry*, **44 (4)**, 687 – 690.

16. K. Germann, M. Kaloga, D. Ferreira, J. P. Marais, and H. Kolodziej (2006), Newbouldioside A–C Phenylethananoid Glycosides from the Stembark of *Newbouldia leavis*. *Phytochem* **67 (8)**, 805 – 811.

17. M. M. Iwu (2000). Handbook of African Medicinal Plants. CRC Press, Inc. London p. 19.

18. V. N. Okafor, U. W. Okafor, R. I. Anyalebechi , M. C. Obiadi , J. N. Obiefuna , C. P. Okonkwo and B. I. Tabugbo (2020), Density effects and its relationship with major flame characteristics of selected fire tolerant trees in South-East Nigeria: a look at oven and sun dried timbers, *J. Chem. Soc. Nigeria*, 45(5), 852 – 862.

19. V. N. Okaor M. C. Obiadi, J. N. Obiefuna (2020), Correlations of major flame characteristics of some fire tolerant trees in South-East Nigeria by coefficient of determination (R^2), *Journal of Scientific Research and Reports*, **26(4)**, 81–98.

20. V. N. Okafor, J. N. Obiefuna, M. C. Obiadi, A. Osuorah, R. I. Anyalebechi and Okafor U. W (2019), Investigating the flammability studies of some fire tolerant trees in South-East Nigeria, *Journal of Basic Physical Research*, **9(2)**, 111-121.

21. V. N. Okafor, I. Enweonye, and P. U. Umennadi (2021), Application of Eigenvalues and Eigenvectors in Correlating Density and Fire Properties of Some Selected Woods in South-East Nigeria. *EJ-ENG, European Journal of Engineering and Technology Research*, **6(6)**, 102-106.

22. I. J. Alinnor (2007), Preliminary phytochemical and antibacterial activity screening of seeds of *Garcenia cola*. *Journal of Chemical Society of Nigeria*, **32(2)**, 41-47.

23. C. E. Ogukwe, E. E. Oguezie, C. Unaegbu, and B. N. Okolue (2004), Phytochemical screening of the leaves of *Sanserieria triyasciata, Journal of Chemical Society of Nigeria*, **29(1)**, 8-10.

24. Natural astringents for healthy and beautiful skin. www.healthstatus.com/health_blog/acne.

ISSN 0795 - 22066

Retrieved 10/2 2017.

25. A. L. Barry and C. Thornsberry (1985), Susceptibility tests diffusion tests procedure, *Journal of Chemical Pathology*, 19:492-500.

26. A.W. Bauer, W.M.M. Kirby, J.C. Sharris, and M. Turck (1966), Antibiotic susceptibility testing by a standardized single disc method, *American Journal of Clinical Pathology*, **45(2)**, 493-496.

27. L. Hakanson (1980), An ecological risk index for aquatic pollution control: a sedimentological approach. *Water Resources*, **14**, 975–1001.

28. D. C. Tomlinson, J. G. Wilson, C. R. Harris and D. W. Jefrey (1980), Problems in assessment of heavy metals in the estuaries and the formation of pollution index. *Helgoland Marine Research*, **33**, 566–575.

29. G. M. S. Abraham and R. J. Parker, (2008), Assessment of heavy metal enrichment factors and the degree of contamination in marine sediments from Tamaki Estuary, Auckland, New Zealand. *Environmental Monitoring Assessment*, **136**, 227–238.

30. Environmental Protection Agency (USEPA) (2016), 2015 Framework for determining a mutagenic mode of action for carcinogenicity: Reviewed draft. Available online: http://epa.gov/osa/mmoaframework/pdfs/ MMOAERD-FINAL-83007.PDF (Accessed 8 August 2016).

31. J. N. Ihedioha, O. T. Ujam, C. O. Nwuche, N. R. Ekere and C. C. Chime (2016), Assessment of heavy metal contamination of rice grains (Oryza sativa) and soil from Adani field, Enugu, Nigeria: estimating the human health risk, human and ecological risk assessment, *An. International Journal*, **22(8)**,1665–1677. https://doi.org/10.1080/ 10807039.2016.1217390.

32. USEPA (2020), Human health risk assessment: risk-based concentration table, Regional Screening Levels (RSLs) Summary Table (TR=1E-06, HQ=1) November 2020. Retrieved from https:// www.epa.gov/risk/regional-screening-levels-rsls-generictables.

33. United States Environmental Protection Agency (USEPA) (2000), Risk-based concentration table. United States Environmental Protection Agency, Philadelphia PA, Washington DC, United States.

34. J. N. Ihedioha, H. O. Abugu, O. T. Ujam, N. R. Ekere (2021), Ecological and human health risk evaluation of potential toxic metals in paddy soil, rice plants, and rice grains (*Oryza sativa*) of Omor rice field, Nigeria, *Environ Monit Assess*, **193**, 620 https://doi.org/10.1007/s10661-021-09386-3

35. L. C. Chien, T. C. Hung, K. Y. Choang, C. Y. Yeh, P. J. Meng, *et al*. (2002), Daily intake of TBT, Cu, Zn, Cd and As for fishermen in Taiwan, *Science of the Total Environment*, **285**, 177–185.

36. C. F. Ibe, A. I. Opara, C. E. Amaobi and B. O. Ibe (2021), Environmental risk assessment of the intake of contaminants in aquifers in the vicinity of a reclaimed waste dumpsite in Owerri municipal, Southeastern Nigeria, *Applied Water Science*, **11**, 24 https://doi.org/10.1007/s13201-020-01355-4

37. C. E. Enyoh and B. O. Isiuku (2021), Determination and human health risk assessment of heavy metals in flood basin soils in Owerri, Southeastern Nigeria, *Chemistry Africa* https://doi.org/10.1007/s42250-020-00171-2.

ISSN 0795 - 22066

38. J. N. Ihedioha, E. O. Ogili, N. R. Ekere and C. C. Ezeofor (2019), Risk assessment of heavy metal contamination of paddy soil and rice (Oryza sativa) from Abakaliki, Nigeria. *Environmental Monitoring and Assessment*, **191**, 350. https://doi.org/10.1007/s10661-019-7491-3.

39. N. Bouayad, K. Rharrrabe, M. Lamhamdi, N.G. Nourouti, F. Sayah (2011). Dietary effect of harmine, a ß –carboline alkaloid, on development, energy reserve and an amylase activity of *Plodia. Interpunctella* Hubner [Lepidoptera:Pyralidae], *Saudi Journal of Biological Sciences*, **1991**, 73-80.

40. P.E. Ghamba, E.B. Agbo, A.F. Umar, D.N. Bukbuk, and L.J. Goje (2012), In invitro antibacterial activity of extracts of crude ethanol, acetone, and aqueous *Garcinia kola* seeds extracts on select clinical isolates, *African Journal of Biotechnology*, **1(6),** 1478-1483.

41. Cardiac glycoside. https://en-wikipedia.org/wiki/cardiac_glycoside

42. T. O. Odugbemi, O. R. Akinsulire, I. E. Aibinu, and P. O. Fabeku (2007), Medicinal plants used for malaria therapy in Ondo state, South West Nigeria, *African Journal Traditional Complementary and Alternative Medicines*, **4(2)**,191-198.

43. D.O. Igboko (1983), Phytochemical Studies on *Garcinia kola* Heckal. M.Sc. Thesis, University of Nigeria, Nsukka. 202.

44. WHO (1989), Evaluation of certain food additives and contaminants, Thirty-third report of the joint FAO/WHO expert committee on food additives, WHO technical series No.807, Geneva, Switzerland?

45. J. Serrano, R. Puupponen-Pimia, A. Daucer, and F. Saura-Calixto (2009), Tannins: Current Hakanson (1980) [25] knowledge of food sources, intake, bioavailability and biological effects. *Molecular and Nutrition Food Research*, **53**, 310-329.

46. O. Timothy and M. Idu (2011), Preliminary phytochemical and in vitro antimicrobial properties of aqueous and methanol extracts of *Icacina trichantha* oliv leaf. *International Journal of Medicinal and Aromatic Plants*, **1(3)**, 184-188.

47. Poliquin Group (2012), Top ten benefits of zinc. Main.Poliquingroup.com/articlesmultimedarticles/articles/812/top _ten_benefits _of_zinc.aspx (Retrieved 8/4/2017).

48. W. H. Liu, J. Z. Zhao, Z. Y. Ouyang, L. Solderland and G. H. Liu (2005), Impacts of sewage irrigation on heavy metal distribution and contamination in Beijing, China, *Environmental International*, **32**, 805–812.

49. P. Zhuang, M. B. McBride, H. Xia, *et al.* (2009), Health risk from heavy metals via consumption of food crops in the vicinity of Dabaoshan mine, South China. *Science of the Total Environment*, **407**, 1551–1561.

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