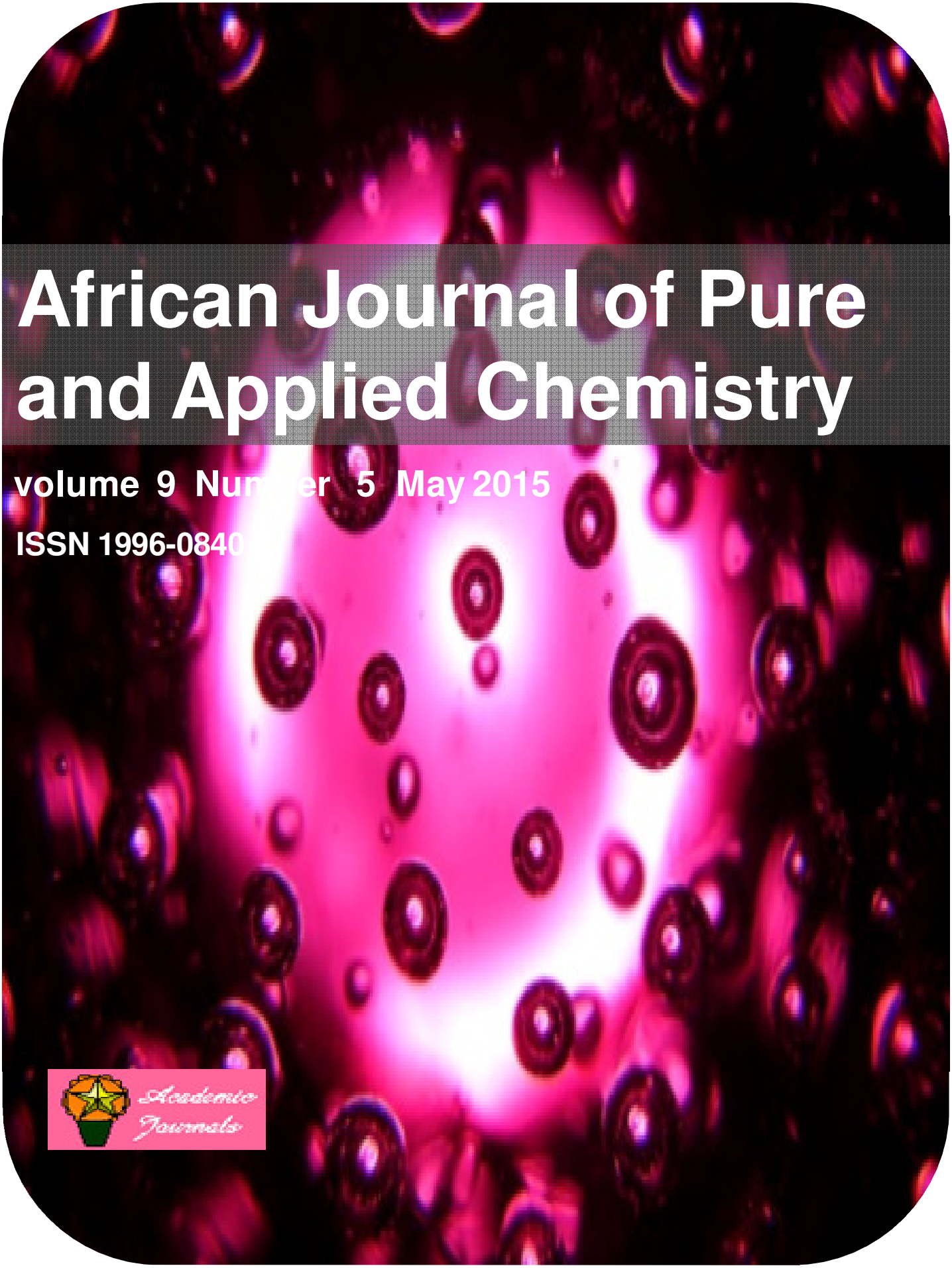


African Journal of Pure and Applied Chemistry

volume 9 Number 5 May 2015

ISSN 1996-0840

Academic Journals

ABOUT AJPAC

The **African Journal of Pure and Applied Chemistry (AJPAC)** is an open access journal that publishes research analysis and inquiry into issues of importance to the science community. Articles in AJPAC examine emerging trends and concerns in the areas of theoretical chemistry (quantum chemistry), supramolecular and macromolecular chemistry, relationships between chemistry and environment, and chemicals and medicine, organometallic compounds and complexes, chemical synthesis and properties, chemicals and biological matters, polymer synthesis and properties, nanomaterials and nanosystems, electrochemistry and biosensors, chemistry and industry, chemistry and biomaterials, advances in chemical analysis, instrumentation, speciation, bioavailability. The goal of AJPAC is to broaden the knowledge of scientists and academicians by promoting free access and provide valuable insight to chemistry-related information, research and ideas. AJPAC is a bimonthly publication and all articles are peer-reviewed.

African Journal of Pure and Applied Chemistry (AJPAC) is published twice a month (one volume per year) by Academic Journals.

Contact Us

Editorial Office: ajpac@academicjournals.org

Help Desk: helpdesk@academicjournals.org

Website: http://www.academicjournals.org/journal/AJPAC

Submit manuscript online http://ms.academicjournals.me/.

Editors

Prof. Tebello Nyokong
Acting Editor
Chemistry Department
Rhodes University
Grahamstown 6140,
South Africa.

Prof. F. Tafesse
Associate Editor
Associate professor
Inorganic chemistry
University of South Africa
South Africa.

Editorial Board

Dr. Fatima Ahmed Al-Qadri
Asst. Professor
Chemistry Department
Sana'a University
Republic of Yemen.

Dr. Aida El-Azzouny
National Research Center
(NRC, Pharmaceutical and
Drug Industries Research Division)
Dokki-Cairo, 12622-Egypt.

Dr. Santosh Bahadur Singh
Department of Chemistry
University of Allahabad
Allahabad, India.

Dr. Gökhan Gece
Department of Chemistry
Bursa Technical University
Bursa, Turkey.

Dr. Francisco Torrens
Institute for Molecular Science
University of Valencia
Paterna Building Institutes
P. O. Box 22085
E-46071 Valencia
Spain.

Dr. Erum Shoeb
Asst. Professor
Department of Genetics
University of Karachi
Karachi-75270
Pakistan.

Dr. Ishaat Mohammad Khan
Physical Research Laboratory
Department of Chemistry
Aligarh Muslim University
Aligarh 202002, India.

Prof. Jean-Claude Bunzli
Department of Chemistry
Swiss Federal Institute of Technology Lausanne
(EPFL)
Institute of Chemical Sciences and Engineering
BCH 1402
CH-1015 Lausanne (Switzerland).

Mrinmoy Chakrabarti
Department of Chemistry,
Texas A&M University
415 Nagle Street, College Station, TX 77840
USA.

Dr. Geoffrey Akien
430 Eisenhower Drive, Apartment B-2,
Lawrence, Kansas 66049,
United States.

Prof. Anil Srivastava
Jubilant Chemsys Ltd.,
B-34, Sector-58,
Noida 201301 (UP),
India.

African Journal of Pure and Applied Chemistry

Table of Contents: Volume 9 Number 5 May, 2015

ARTICLES

Research Articles

academicJournals

Vol. 9(5), pp. 81-90, May, 2015
DOI: 10.5897/AJPAC2015.0619
Article Number: 4A9E2A552894
ISSN 1996 - 0840
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJPAC

African Journal of Pure and Applied Chemistry

Full Length Research Paper

Determination of phenylethyl alcohol by reversed-phase high-performance liquid chromatography (RP-HPLC) in Budesonide nasal spray

Reza Hosseini[1], Fereshteh Naderi[2]* and Saman Ahmad Nasrollahi[3]

[1]Department of Chemistry, Faculty of Science, Shahr-e-Qods Branch Islamic Azad University, Tehran, Iran.
[2]Department of Chemistry, Shahr-e-Qods Branch Islamic Azad University, Tehran, Iran.
[3]Formulations Lab., Center for Research and Training in Skin Diseases and Leprosy, Tehran University of Medical Sciences, Tehran, Iran.

Received 2 March, 2015; Accepted 14 April, 2015

Phenylethyl alcohol is used as an antimicrobial preservative in many pharmaceutical products, especially nasal sprays. A simple and accurate reverse phase high performance liquid chromatographic method was developed to assay of phenylethyl alcohol in budesonide nasal spray preparations. A waters C18 symmetry column chromatographic system (150 × 4.6 mm, 5 µm particle size) was performed with a 50:50 (%V/V) mixture of water and acetonitrile as a mobile phase. The detection of the phenylethyl alcohol was carried out at 220 nm and flow rate was employed 1.0 ml/min. The retention time of phenylethyl alcohol was about 2.8 min. Linearity was established in the concentration range of 173.28 to 259.92 mg/ml (80 to 120% of the target concentration), with a regression coefficient of 0.9991. Specificity was tested in the presence of placebo; no interference was detected at the retention time of phenylethyl alcohol. The results of the analysis were validated statistically and recovery percentage studies confirmed the accuracy and precision of the proposed method.

Key words: Phenylethyl alcohol, budesonide, nasal spray, reversed-phase high-performance liquid chromatography (RP-HPLC), preservative.

INTRODUCTION

Allergic rhinitis is a common disease and refers to inflammation of the nasal passages including sneezing, itching, nasal congestion and runny nose. Intranasal corticosteroids are among the most effective treatments for permanent allergic rhinitis. Some individuals unable to tolerate aerosols may prefer an aqueous nasal spray (Mygind, 1993).

Budesonide, the active component of budesonide nasal spray is a corticosteroid designated chemically as (RS)-11β, 16α, 17, 21-Tetrahydroxypregna-1, 4-diene-3, 20-

*Corresponding author. E-mail: fnaderi2@yahoo.com, Tel/Fax: (+9821) 46896000.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

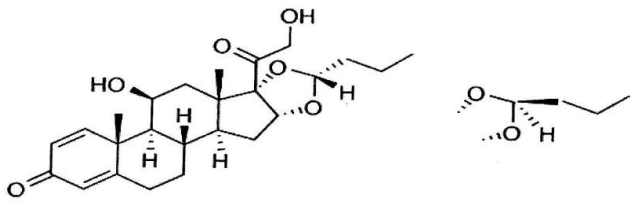


Figure 1. The structural formula of budesonide.

dione cyclic 16, 17-acetal with butyraldehyde. Budesonide is provided as a mixture of two epimers (22R and 22S). The empirical formula of budesonide is $C_{25}H_{34}O_6$ and its molecular weight is 430.5. Its structural formula was shown in Figure 1.

Budesonide is a white to off-white, tasteless, odorless powder that is practically insoluble in water and in heptanes, sparingly soluble in ethanol, and freely soluble in chloroform. Its partition coefficient between octanol and water at pH 7.4 is 1.6×10^3. Budesonide is an anti-inflammatory corticosteroid that exhibits potent glucocorticoid activity and weak mineralocorticoid activity. In standard *in vitro* and animal models, budesonide has approximately a 200-fold higher affinity to the glucocorticoid receptor and a 1000-fold higher topical anti-inflammatory potency than cortisol. As a measure of systemic activity, budesonide is 40 times more potent than cortisol when administered subcutaneously and 25 times more potent when administered orally in the rat thymus involution assay (Rice-Thomas et al., 2009).

Antimicrobial preservatives are included in preparations to kill or inhibit the growth of microorganisms inadvertently introduced during manufacture or use. They are used in sterile preparations such as eye-drops and multidose injections to maintain sterility during use and in cosmetics, foods, and non-sterile pharmaceutical products such as oral liquids, creams, inhalations and nasal sprays to prevent microbial spoilage. The choice of a suitable preservative for a preparation depends on pH, compatibility with other ingredients, the route, dose and frequency of administration, partition coefficients with ingredients and containers or closures, degree and type of contamination, concentration required, and rate of antimicrobial effect (Thomas et al., 1989).

Phenylethyl alcohol is an excipient of budesonide nasal spray. It is an antimicrobial preservative designated chemically as 2-Phenylethanol. The empirical formula of phenylethyl alcohol is $C_8H_{10}O$ (Figure 2) and its molecular weight is 122.17. Phenylethyl alcohol is a clear, colorless liquid with an odor of rose oil. It has a burning taste that irritates and then anesthetizes mucous membranes (Rowe et al., 2009; O'Neil et al., 2001). Phenylethyl alcohol is very soluble in alcohol, in fixed oils, in glycerin, and in propylene glycol, and sparingly soluble in water and slightly soluble in mineral oil Franson et al., 2012).

Phenylethyl alcohol in relatively low concentrations

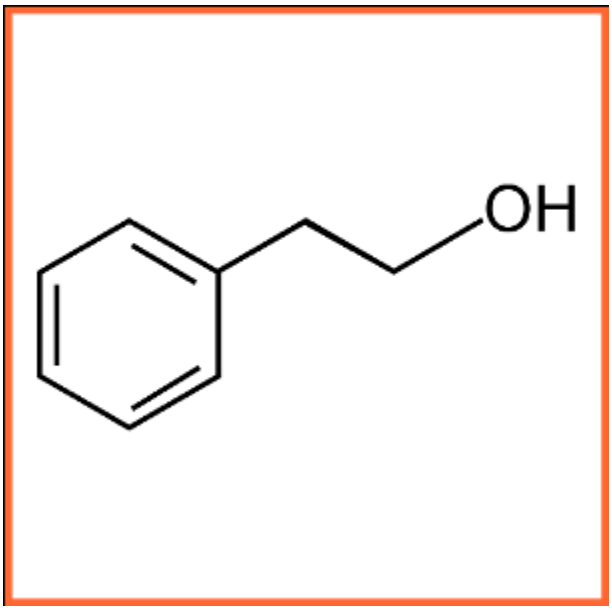


Figure 2. The structural formula of phenylethyl alcohol.

(1:400) exerts an effective inhibitory action on Gram-negative bacteria and may thus be used for differential inhibition (Lilley and Brewer, 1953; Hodges et al., 1996). Assay and detection of phenylethyl alcohol in nasal sprays is one of the important experiments during manufacturing process in quality control laboratory. Therefore the aim of this study was finding a fast and valid measurement method to assess phenylethyl alcohol in budesonide nasal sprays.

High performance liquid chromatography (HPLC) is one of the most powerful analysis methods. In recent years the use of reversed-phase high-performance liquid chromatography (RP-HPLC) method for determination of drug substances is very common and HPLC instruments and RP-HPLC solvents are available in most pharmaceutical laboratories. For this reasons, we developed a RP-HPLC method for determination of phenylethyl alcohol in Budesonide nasal spray and similar formulations which this analysis method is simple, fast, short response time, cheap price, with high accuracy and high precision. This analysis method was fully validated and can be done easily in any laboratories.

MATERIALS AND METHODS

HPLC grade acetonitrile was procured from Merck Company (Germany), pure standard of phenylethyl alcohol (99.9 % w/w) was obtained from LGC Company (England) and HPLC grade water was prepared by using Millipore Milli Q plus purification system (USA). The 0.45 µm nylon filter was obtained from Millipore Company (USA) and L1 columns were procured from both Waters Company (USA) and Agilent Company (USA). All other chemicals were analytical grade and commercially available.

Chromatography (Waters HPLC system, USA) was performed

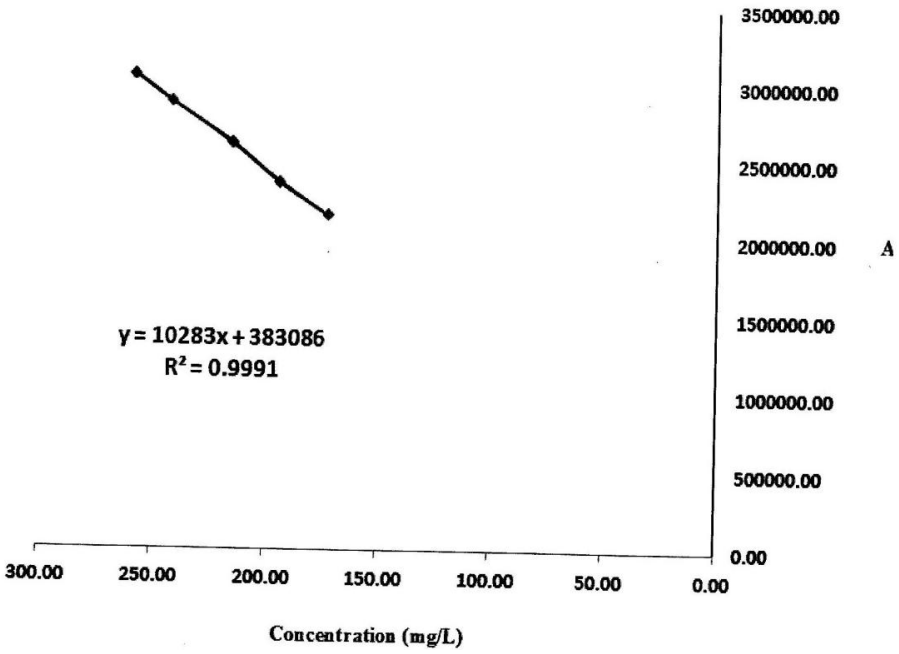


Figure 3. Linearity curve of phenylethyl alcohol in budesonide nasal spray preparation.

with a 1525 separation module through inherent manual injector jointed to 2487 UV detector and also a 2695 separation module with inbuilt auto injector and 2996 photodiode array detector. Waters C18 symmetry column (150×4.6 mm, 5 µm particle size) and Agilent C18 column were used for chromatographic separation under isocratic elution. Detection was carried out using an UVspectrophotometric detector at 220 nm and Waters Breez and Empower software was used. The mobile phase was a 50:50 (% v/v) mixture of prepared water and acetonitrile. Mobile phase was sonicated and degassed before use. The flow rate of mobile phase was adjusted at 1.0 ml/min. The column temperature was maintained at ambient conditions. The injection volume was 20 µl and total run time was 5 min. The phenylethyl alcohol was identified by retention time of the standard phenylethyl alcohol peak. Also in specificity test, phenylethyl alcohol peak was identified against standard compound peak in the presence of placebo.

Validation of the method

Based on previous and similar chromatographic methods, the best system for determining of phenylethyl alcohol was selected (Harris, 1991; Skoog et al., 1991; Moffat et al., 2011). The method is intended to assay phenylethyl alcohol in budesonide nasal spray during analytical method validation. The method was validated, in accordance with ICH guidelines (Authors Group, 2005) and other similar works (Rao et al., 2010; Blanco et al., 1999). All validation factors such as linearity, specificity, accuracy, precision, repeatability, reproducibility and robustness were assessed.

RESULTS

Linearity

Linearity was obtained with the concentration range of 173.28 to 259.92 mg/L for phenylethyl alcohol. Linearity was performed with different dilutions. Calibration solutions were 80 to 120% of the target concentration. Calibration graph was plotted on the basis of analysis of calibration solutions. The coefficient of regression was obtained 0.9991 and the slop of 10283 was achieved (Figure 3). Standard stock solution of phenylethyl alcohol (2.166 g/L) was prepared by dissolving it in water. From this stock, concentrations of 173.28, 194.94, 216.60, 243.67, 259.92 mg/L were prepared in acetonitrile. Each solution was injected three times except that the target solution (216.60 mg/L) was injected six times. The results are shown in Table 1 and Figure 3.

Specificity

Specificity was tested against standard compound and against potential interferences in the presence of placebo. As Figures 4, 5 and 6 depict, no interference was detected at the retention time of phenylethyl alcohol in placebo solution.

Accuracy

Accuracy was determined by the two methods including: 1) Spiking the phenylethyl alcohol standard in placebo. 2) Using linearity curve. Standard stock solution of phenylethyl alcohol (2.1508 g/L) was prepared in water. 1.0 ml of this solution was transferred into 10 ml volumetric flask and diluted with acetonitrile to achieve a

Table 1. Linearity results of phenylethyl alcohol in budesonide nasal spray preparation.

Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
173.28	2.810 2.811 2.811	2168590 2165472 2166079	2166713.67	0.02	0.08
194.94	2.811 2.810 2.811	2372223 2376421 2376063	2374902.33	0.02	0.10
216.60	2.811 2.812 2.812 2.811 2.813 2.812	2628468 2629975 2625099 2626837 2626590 2626687	2627276	0.03	0.06
243.67	2.812 2.812 2.812	2876793 2891142 2889632	2885855.67	0.00	0.27
259.92	2.811 2.811 2.810	3052790 3055870 3050643	3053101	0.02	0.09

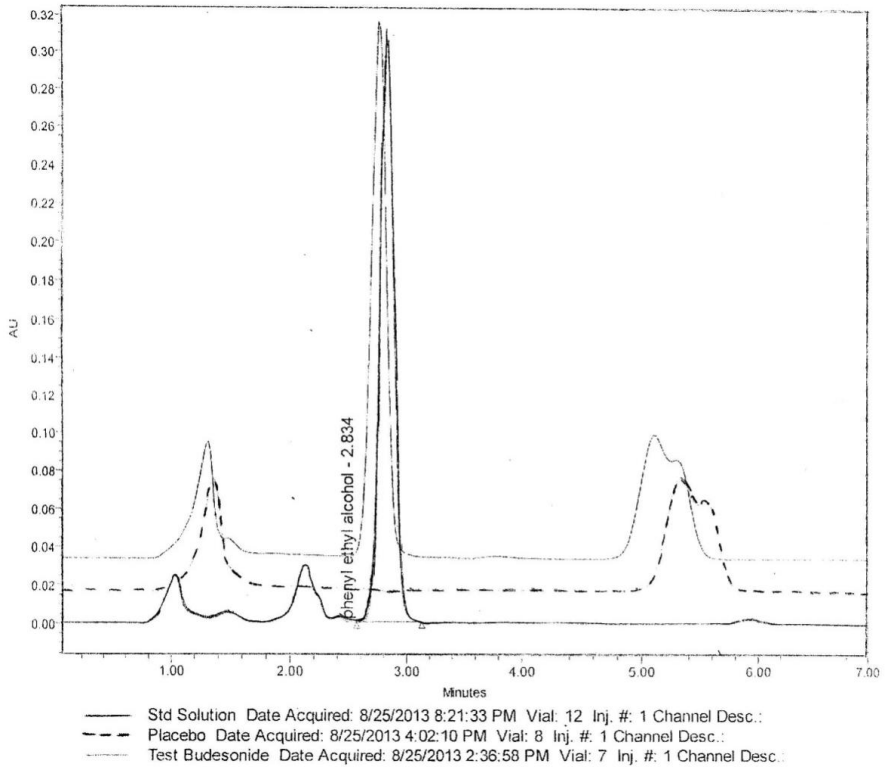


Figure 4. Chromatograms of Standard solution, Test Solution and Placebo solution (with PDA detector).

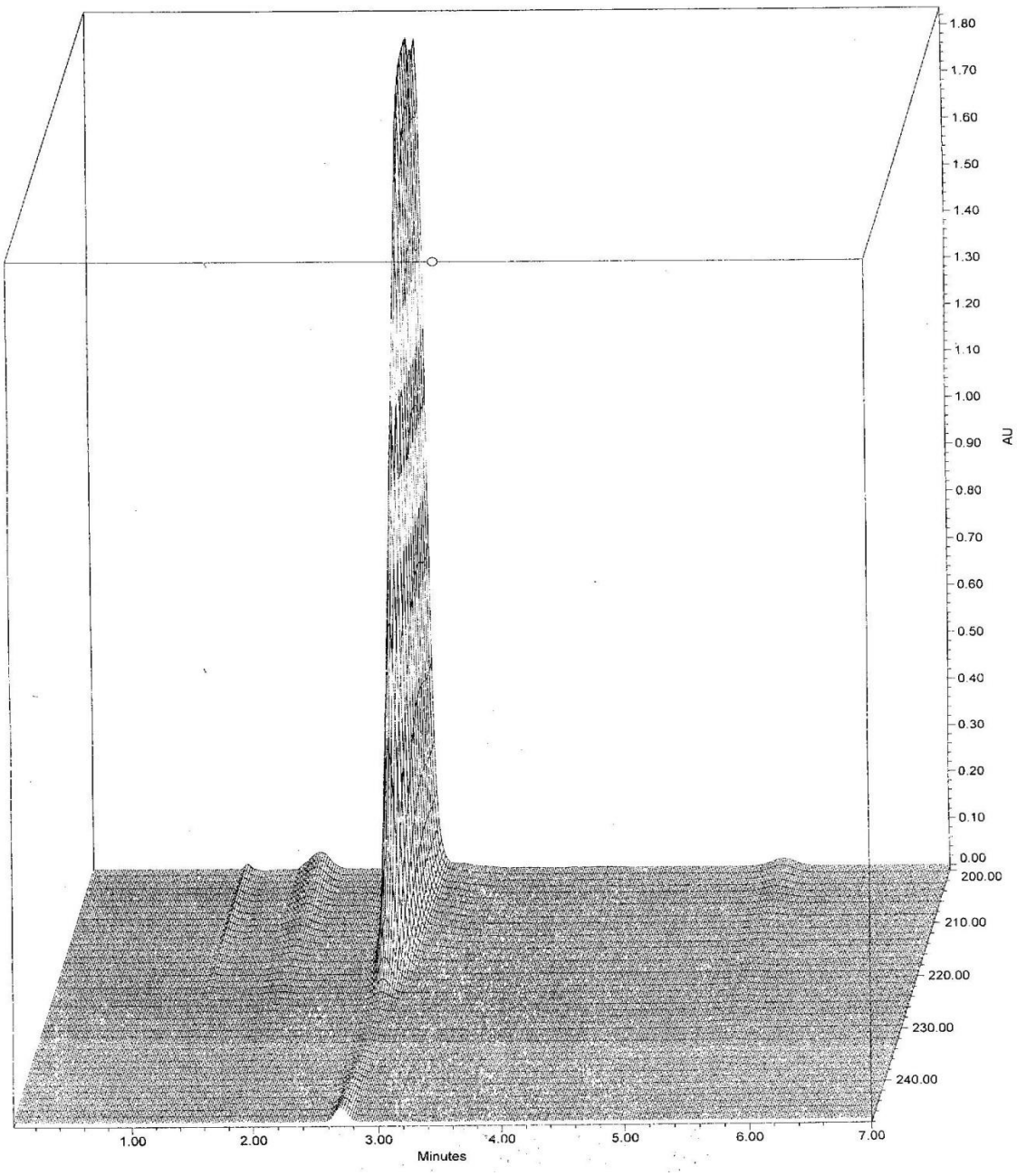


Figure 5. Three-dimensional chromatogram of phenylethyl alcohol peak in Standard solution (with PDA detector).

final concentration of 215.08 mg/L. Concentrations of 187.5, 206.25, 176.25 mg/L with acetonitrile were prepared from budesonide nasal spray.

Spiking the phenylethyl alcohol standard in placebo

Accuracy was evaluated by spiking the phenylethyl alcohol standard in placebo at three different concentrations level and were calculated the recovery percentages with external standard method. Results are presented in Tables 2 and 3. Recovery percentages were in the range of 98.0 to 102.0% that show this method has suitable accuracy.

Using linearity curve

Accuracy was estimated by this method at three different concentration levels and recovery percentages were

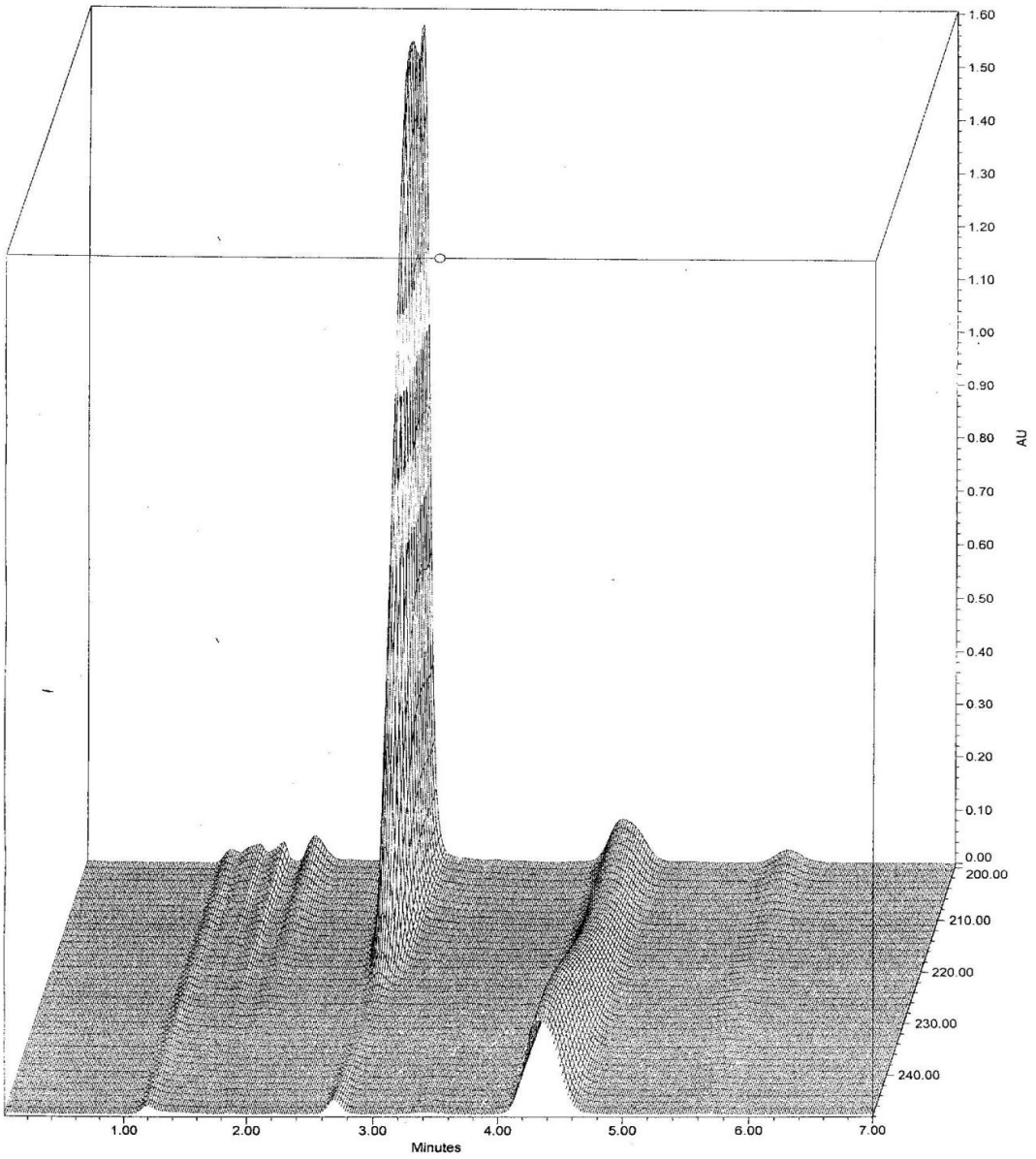


Figure 6. Three-dimensional chromatogram of phenylethyl alcohol peak in Sample solution of budesonide nasal spray preparation (with PDA detector).

calculated with use linearity curve. In this method concentrations were obtained by a linear equation and recovery percentages were in the range of 98.0 to 102.0% that demonstrate this method has suitable accuracy. These results are represented in Table 4. Spiking in placebo and using linearity curve overall confirm which accuracy of this method is in the series of very high-quality.

Precision

Precision was studied in the three levels including: Repeatability (Intra-assay precision), ruggedness and solution stability (Intermediate precision) and reproducibility. Standard stock solution of phenylethyl alcohol (2.1508 g/L) was prepared in water. 1.0 ml of this solution was moved to 10 ml volumetric flask and diluted with acetonitrile to achieve a final concentration of 215.08 mg/L. Concentrations of 200, 250 and 220 mg/L with acetonitrile were prepared from budesonide nasal spray.

Repeatability

Repeatability was studied at three different concentration

Table 2. Chromatographic results of accuracy test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
		2.834	2300932			
	187.5	2.910	2300298	2300642.00	1.38	0.01
		2.892	2300696			
		2.854	2497974			
Sample	206.25	2.834	2502115	2500475.00	0.35	0.09
		2.843	2501336			
		2.835	2177020			
	176.25	2.843	2164883	2169686.67	0.15	0.30
		2.837	2167157			
		2.832	2609245			
Standard	215.08	2.832	2612129	2609373.33	0.02	0.10
		2.831	2606746			

Table 3. Accuracy results of phenylethyl alcohol in budesonide nasal spray preparation by external standard method.

True concentration (mg/L)	Found concentration (mg/L)	% Recovery
187.5	189.63	101.14
206.25	206.10	99.93
176.25	178.84	101.47

Table 4. Accuracy results of phenylethyl alcohol in budesonide nasal spray preparation by linearity curve.

True concentration (mg/L)	Found concentration (mg/L)	% Recovery
187.5	186.48	99.46
206.25	205.91	99.84
176.25	173.74	98.58

levels and relative standard deviations of results were calculated. Results in Tables 5 and 6 were found less than 2.0%.

Ruggedness and solution stability

The ruggedness of the method was studied on three different days with different analysts. The relative standard deviations of results were found less than 2.0%. To demonstrate the stability of both standard and sample solutions during analysis, both solutions were analyzed over a period of 48 h at room temperature. The results showed that for all solutions, the retention times and peak areas of phenylethyl alcohol remained almost unchanged (RSD<2.0%) which indicating that no significant degradation occurred within this period. Both solutions were stable for at least 48 h. These results are presented in Tables 7 and 8.

Reproducibility

The reproducibility of method was studied in the two

Table 5. Chromatographic results of repeatability test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
Sample	200	2.779	2316501	2318291	0.05	0.09
		2.780	2317825			
		2.777	2320547			
	250	2.776	2940676	2963738	0.04	0.68
		2.775	2971893			
		2.777	2978645			
	220	2.775	2620006	2622414.67	0.06	0.08
		2.772	2624101			
		2.773	2623137			
Standard	215.08	2.843	2632891	2632455.67	0.23	0.14
		2.832	2628565			
		2.831	2635911			

Table 6. Repeatability results of phenylethyl alcohol in budesonide nasal spray preparation by external standard method.

Sample concentration (mg/L)	Result (%)	RSD (%)
200	94.71	
250	96.86	1.47
220	97.39	

Table 7. Chromatographic results of ruggedness test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
Sample	200	2.761	2395008	2396151.67	0.00	0.06
		2.761	2397899			
		2.761	2395548			
	250	2.761	2967927	2978031	0.04	0.30
		2.763	2983895			
		2.763	2982271			
	220	2.813	2617292	2614569	0.02	0.09
		2.813	2612827			
		2.814	2613588			
Standard	215.08	2.834	2626307	2627129	0.05	0.14
		2.832	2623848			
		2.831	2631232			

Table 8. Ruggedness results of phenylethyl alcohol in budesonide nasal spray preparation by external standard method.

Sample concentration (mg/L)	Result (%)	RSD (%)
200	98.09	
250	97.52	0.42
220	97.30	

Table 9. Chromatographic results of reproducibility test.

	Concentration (mg/L)	R.t	Area	Mean (Area)	% RSD (R.t)	%RSD (Area)
Sample	200	2.760	2377528	2382569.33	1.02	0.19
		2.762	2384364			
		2.810	2385816			
	250	2.766	2989100	2987751.33	0.39	0.10
		2.763	2989812			
		2.783	2984342			
	220	2.813	2618586	2618884.67	0.00	0.01
		2.813	2618922			
		2.813	2619146			
Standard	215.08	2.834	2627913	2626403	0.05	0.06
		2.832	2626417			
		2.831	2624879			

Table 10. Reproducibility results of phenylethyl alcohol in budesonide nasal spray preparation (in other laboratory with other Waters HPLC instrument).

Sample concentration (mg/L)	Result (%)	RSD (%)
200	97.56	
250	97.87	0.21
220	97.48	

different laboratories. The percentage relative standard deviations of results with other Waters HPLC Instrument and in other laboratory were found less than 2.0%. The results are given in Tables 9 and 10.

Robustness

The robustness of the method was determined by making slight changes in the chromatographic conditions that is, mobile phase ±5%, flow rate ±0.1 ml/min (Woolfson et al., 2014). Also this method was done with another C18 column (150×4.6 mm, 5 µm, Agilent Company), and finally similar results were obtained.

DISCUSSION

The purpose of this study was development a method to determination of phenylethyl alcohol in budesonide nasal spray and other similar nasal anti allergic formulations. The mixture of water and acetonitrile in different ratios was examined as a mobile phase and lastly a mixture of water and acetonitrile in the ratio of 50:50 (V/V) and flow

rate of 1.0 ml/min was selected. The optimum wavelength for detection was considered at 220 nm (because of no interfering and suitable shape). After obtaining these final conditions of the chromatographic system, validation of the method was performed.

(i) Linearity was recognized in the concentration range of 173.28 to 259.92 mg/ml (80 to 120% of the target concentration) with a regression coefficient of 0.9991.
(ii) Specificity was experienced in the presence of placebo; no interference was detected at the retention time of phenylethyl alcohol.
(iii) Accuracy was determined by the two technique of spiking and linearity curve. Recovery percentages were calculated and results were in the range of 98.0 to 102.0%.
(iv) Precision was studied in the three levels including; repeatability, ruggedness and reproducibility.

Percentage relative standard deviations of the results were calculated that were less than 2.0%. The results of the analysis were validated statistically and confirmed the accuracy and precision of the proposed method.
We concluded that proposed RP-HPLC method for determination of phenylethyl alcohol in budesonide nasal spray is simple, precise, specific, and highly accurate and this method is very less time consuming in quality control laboratories. So, this method can definitely be used in phenylethyl alcohol drug substance analysis and determination of phenylethyl alcohol in budesonide nasal spray and other similar nasal anti-allergic formulations such as Fluticasone nasal spray, Beclometasone nasal spray, Mometasone nasal spray and etc. The advantages of this method over other old methods are short retention time for determination of phenylethyl alcohol (about 2.7 min), simple mobile phase, economical and practical procedure to assay phenylethyl alcohol in other similar pharmaceutical products.

Conflict of Interest

The authors of this paper declared no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Leila Gholampour who helped sympathetically and also special thanks to Dr. Lida Khalafi and Dr. Mohammad Rafiei because of their advices on writing this paper. In the end, authors are appreciated from Research and Development Department of Jaber Ebne Hayyan pharmaceutical Company.

REFERENCES

Mygind N (1993). Glucocorticosteroids and rhinitis Allergy. Eur. J. Allergy Clin. Immunol. 48(7):476–490.
Rice Thomas F, Santarpia D, Caine CY, Lagow B, Struble S, Shenouda N (2009). Physicians Desk Reference® (PDR®) 63th editions: 654.
Thomas WJ, Wade A, Reynolds James EF (1989). Martindale 29 th editions: 1355.
Rowe CR, Sheskey JP, Quinn EM (2009). Handbook of Pharmaceutical Excipients, 6th editions: 490-491.
O'Neil Maryadele J, Smith A, Heckelman Patricia E, Obenchain Jr JR, Gallipeau Jo Ann R, Arecca MAD (2001). The Merck Index, An Encyclopedia of chemicals, Drugs, and biologicals, Volume 2, 14 th edition, published by Merck Research Laboratories, Whitehouse station: 1296.
Franson TR, Bravo RH, Courtney JE, Demars SS, Kirking DM, Williams RL (2012). United State Pharmacopeia 35 (National formulary 30), 1:1136.
Lilley BD, Brewer JH (1953). The selective antibacterial action of phenylethyl alcohol. J. Am. Pharm. Assoc. Sci. 42:6-8.
Hodges NA, Denyer SP, Hanlon GW, Reynolds JP (1996). Preservative efficacy tests in formulated nasal products: reproducibility and factors affecting preservative activity. J. Pharm. Pharmacol. 48:1237-1242.
Harris CD (1991). Quantitative chemical analysis, 3th editions, W. H. Freeman and Company, New York: 666-680.
Skoog AD, West MD, Holler JF (1991). Fundamentals of analytical chemistry, 6th editions, Saunders college publishing, Toronto: 712-737.
Moffat CA, Osselton DM, Widdop B (2011). Clarke's analysis of drugs and poisons, 4th editions, published by Pharmaceutical Press, London: pp. 597-598.
Authors Group (2005). ICH Guidelines Q2 (R1) – Validation of Analytical Procedures: Text and Methodology: pp. 1-13.
Rao KLN, Reddy KP, Babu KS, Raju KS, Rao KV, Shaik JV (2010). Simultaneous estimation of Fluticasone propionate, Azelastine hydrochloride, Phenylethyl alcohol and Benzalkonium chloride by RP-HPLC method in nasal spray preparations. J. Res. Pharm. Sci. 1(4):473-480.
Blanco M, Serrano D, Bernal JL (1999). UV-spectrophotometric determination of beclometasone dipropionate and phenylethyl alcohol in a nasal spray by inverse least-squares. Talanta 50(3):527-532.
Woolfson D, Fenton-May VI, Buckton G, Cairns DB, Cook GD, Davidson A, Duffy TD, Goddard C, Helliwell K, Horder R, Matthews BR, Tsang L (2014). British Pharmacopoeia, Appendix III, Volume 5: V-A189-190.

academicJournals

Vol. 9(5), pp. 91-97, May, 2015
DOI: 10.5897/AJPAC2015.0622
Article Number: D34CF8552895
ISSN 1996 - 0840
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJPAC

African Journal of Pure and Applied Chemistry

Full Length Research Paper

Phytochemical composition, antioxidant and anti-nutritional properties of root-bark and leaf methanol extracts of *Senna alata* L. grown in Nigeria

Abubakar I.*, Mann A. and Mathew J. T.

Department of Chemistry, Federal University of Technology, P. M. B. 65, Minna, Niger State, Nigeria.

Received 7 April, 2015; Accepted 8 May, 2015

***Senna alata* L. has been ethnobotanically used extensively in traditional medicines for the treatment of a variety of diseases such as skin problems, arthritis, HBP (high blood pressure), and laxative or purgative. The phytochemical, anti-nutritive and antioxidant activities of the leaf and root bark of *Senna alata* L. grown in Bida Niger State, Nigeria were determined using standard analytical methods. Phytochemical screening of the flower and seed of the plant revealed the presence of flavonoids, phenols, saponins, tannins, alkaloid and anthraquinone. Steroid and cardiac glycosides, were slightly present, while resins were absent. The metabolites present were quantitatively determined with alkaloid contents of 14.09±0.50 and 15.89±0.72, saponin 40.57±0.57 and 33.02±0.07, flavonoid 42.28±0.90 and 36.52±0.38, tannin 59.48±0.50 and 44.38±0.72, and phenol 7.84±0.49 and 9.91±0.68 mg/100 g for leaf and root bark respectively. These results confirm that the metabolites obtained from the two parts of this plant were within the range of toxicity levels according to World Health Organization safe limits. The results of anti-nutritional factors revealed oxalate contents of 7.84±0.74 and 9.91±0.62, cyanide content 13.04±0.09 and 21.69±0.11 and phytate content 15.07±0.58 and 12.44±0.31 mg/100 g for leaf and root bark respectively. The values of anti-nutritional factors obtained from this work show that they may not pose any effects based on their toxicity levels and as recommended by World Health Organization. The major components of the chemical compounds deduced from GC/MS for the two parts of this plant investigated revealed the presence of α-d-mannofuranoside (53.35%), oleic acid (12.30%), β-d-glucopyranoside (12.59%), β-d-mannofuranoside (22.41%), n-hexadecenoic acid (5.73%), 1,2,3-propanetriol (21.54%), α-d-glucopyranoside (16.41%) and oleic acid (14.65%). Thus, if properly domesticated and produced in commercial quantities, this plant will serve as a source of bioactive agents for pharmaceuticals.**

Key words: Antioxidant, phytochemical, anti-nutritional, leaf, root bark and *Senna alata*.

INTRODUCTION

Plants have been used for medicinal purposes over the years, which have provided mankind with a source of essentials of life such as food, medicine and raw materials for clothing and shelter (Midawa et al., 2010).

*Corresponding author. E-mail: abubakis@mtnnigeria.net
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

According to World Health Organization (2002), traditional medicine refers to health approaches, practices, beliefs and knowledge incorporating animals, plants, and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, prevent and diagnose illnesses. Over 80% of world population uses traditional medicine to cure various diseases (WHO, 2002). The major ingredients are obtained from medicinal plants. It has been discovered that majority of modern medicine are plant-derived therapeutic agents. This could be attributed to the fact that many plants contain a variety of phytochemicals, which have found very important applications in the field of human medicine. Natural products play a dominant role in the development of novel drug- leads for the treatment and prevention of disease (Newman et al., 2003). Reactive oxygen species (ROS) such as superoxide radical, hydroxyl radical, singlet oxygen, and hydrogen peroxide are produced in the body during normal metabolism or on exposure to exogenous factors. These reactive species can initiate deterioration of biomolecules such as proteins, lipids, carbohydrates and nucleic acids and are implicated in several diseases such as ageing, atherosclerosis, inflammatory injury, cancer, cardiovascular disease, neurological disorders etc. Oxidative stress results, when the balance between the generation of ROS and antioxidant defense system of the body is disturbed. Cells have innate defense system which protects against the adverse effects caused by these ROS and includes enzymatic and non-enzymatic defense. However, during pathophysiological conditions, there is an extra need for antioxidants from exogenous sources. Synthetic antioxidants have been suspected to cause or promote negative health effects. Hence, there is a need for development of safer antioxidants particularly from natural sources. Many studies have demonstrated the efficacy of plant derived products as antioxidants against various diseases induced by these free radicals (Koleva et al., 2000). It has been shown that the antioxidant nature of plants is mainly attributed to phenolic compounds, such as flavonoids and phenolic acids (Pietta, 2000). *Senna alata* L. has been ethnobotanically used extensively in traditional medicines for the treatment of a variety of diseases such as skin problems, arthritis, high blood pressure (HBP) and laxative using its organs. Therefore, there is need to investigate these plant organs for the bioactive compounds to be used for therapeutic purposes.

MATERIALS AND METHODS

Collection, identification and preparation of plant material

Leafy plant of *S. alata* was obtained from Edokota forest along Bida-Zungeru road, Bida, Niger State, Nigeria. The identity was confirmed by plant taxonomist from the National Institute of Pharmaceutical Research Development, Idu-Abuja where a voucher specimen was deposited with Herbarium No.1369. The samples (leaf and root bark) collected from the experimental sites were washed with distilled water to remove impurities and dried at room temperature. These were then grind into uniform powder manually. It was then sieved, weighed, bottled, labelled and used for laboratory analysis.

Extraction of the plant extracts

Powdered flower and seed of *S. alata* were extracted with 70% aqueous methanol at room temperature. The extract solution of each sample was filtered, and the solvent was evaporated under reduced pressure at 35°C (Figure 1).

Qualitative phytochemical screening of the samples

Phytochemical screening procedures carried out were adopted from Mann (2014), where tannins, saponins, steroids, alkaloids, cardiac glycoside, terpenoids and flavonoids were determined.

Quantitative phytochemical analysis of the samples

Standard analytical methods were used for the quantitative phytochemical analysis of these samples (Edeoga et al., 2005). Tannins and saponins were determined using standard method of Onwuka (2005), while flavonoids and alkaloids were determined using standard method as described by Harborne (1989) and the total phenolic content was estimated using the modified Folin-Ciocalteu photometric method by Schuler (1990).

Anti-nutritional properties of the samples

Oxalate and cyanide contents were determined using the method of Day and Underwood (1986). Phytate content was determined by the method described by Wheeler and Ferrel (1971).

Antioxidants activities of the samples

The ferric reducing antioxidant power (FRAP) assay was done according to Benzie and Strain (1996) with some modifications while total phenolics of various fractions of plant were determined by reported method of Valentao et al. (2002).

GC/MS analysis of the samples

GC-MS analysis was carried out on a Shimadzu (Kyoto, Japan) GC-MS model QP 2010 at National Research Institute for Chemical Technology, Zaria, according to the EN 14103 standard method (Adams, 2007; Orishadipe et al., 2010). The GC column oven temperature (70°C), injecting temperature (250°C), flow control mode (linear velocity), total flow (40.8 ml/min) column flow (1.80 ml/min), pressure (116.9 kpa), linear velocity (49.2 cm/s) and purge flow (3.0 ml/min) were employed for this analysis. A sample volume of 8.0 µl was injected using split mode (split ratio of 20:0). The peak area, that is, the % amount of every component was calculated by comparing its average peak area to the total areas. Software was used to handle mass spectra and chromatogram.

Identification of components from the samples

Interpretation of mass spectrum GC-MS was conducted by

Figure 1. Matured strands of *S. alata* with leaves (A. Mann).

Table 1. Qualitative phytochemical evaluation of methanol extracts of *S. alata* leaf and root bark.

Chemical constituents	Chemical test	Methanol leaf extract	Methanol root bark extract
Alkaloids	Hager's test	+	+
	Wagner's test	–	–
Tannins	Tannin test	+	+
Anthraquinone	Anthraquinone test	+	+
Saponins	Foam's test	+	+
Glycosides	Borntrager's test	+	+
	Liebermann Buchard's test	+	+
	Legal's test	+	+
Triterpenes and Phytosterols	Salkowski's test	+	+
	Lieberman Buchard's test	+	+
Phenols	Ferric chloride tests	+	+
Flavonoids	Sodium hydroxide test	+	+
	Lead acetate test	+	+
	Shinoda test	–	–
Resins	Acetone-water test	–	–

+ = Indicates presence, − = Indicates absence.

comparing the database peaks of National Institute of Standard and Technology (NIST) library with those reported in literature, the mass spectra of the peaks with literature data (Stein et al., 2002). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Component relative percentages were calculated based on GC peak areas without using correction factors. The name, molecular weight and structure of the components of the test materials were ascertained.

Statistical analysis

All the experiments were conducted in triplicate unless stated otherwise and statistical analysis of the data was performed by analysis of variance (ANOVA), using SPSS 11.0 for Windows software. A probability value of difference $p \leq 0.05$ was considered to denote a statistically significance. All data were expressed as mean values ± standard deviation (SD).

RESULTS AND DISCUSSION

The results of qualitative analysis of the crude methanolic extract of two parts of *S. alata* shown in Table 1 revealed the presence of tannins, flavonoids, terpenoids, saponins, alkaloid, glycosides, anthraquinone, which are the basis

Table 2. Quantitative evaluation of methanol extracts of *S. alata* leaf and root bark (mg/100 g).

Chemical constituents	Samples	
	Leaf	Root bark
Alkaloids	14.09±0.50[b]	15.89±0.72[a]
Saponins	40.57±0.55[a]	33.02±0.07[b]
Flavonoids	42.28±0.90[a]	36.52±0.38[b]
Tannins	59.48±0.50[a]	44.38±0.72[b]
Phenols	7.84±0.49[b]	9.91±0.68[a]

Values are means ±SD of three determinations, different superscripts along the same row are significantly different (p≤0.05).

of therapeutic potentials of medicinal plants. Similar results were reported for *Senna obtusifolia* by Essiett and Bassey (2013) where the presence of saponins, tannins, alkaloids, terpenoids, anthraquinone and a trace of steroids were reported. The presence of tannins as reported in this work may be the cause of lowering of available protein by antagonistic competition and can therefore elicit protein deficiency syndrome "Kwashiokor" (Maynard, 1997). Saponin may be responsible for its anti-yeast, anti-fungal, antidote, antimicrobial and anti-inflammatory activities. It is also believed that the role of saponin is to protect plant against attack by potential pathogens (Sparg et al., 2004). Flavonoids which are also known as vitamin p or plan modifier, elicit a wide range of therapeutic activities as antihypertensive, antirheumatism as well as antimicrobial as identified with flavonoids (Veerachari and Bopaiah, 2011). Essiett et al. (2010) reported that many plants containing flavonoids have diuretic and antioxidant properties. The leaf and root bark of this plant can equally be used accordingly; glycosides were detected in the extracts and this class of compound has been found useful in the treatment of asthma (Trease and Evans, 1989; Evans, 2002). Steroids were also found and their pharmaceutical importance might hinge on their relationship with such compounds as sex hormones (Bell, 2007). Glycosides were detected in the leaf and root bark of *S. alata.* Glycoside has been used for over two centuries as stimulant in cases of cardiac failure and diseases (Taiwo et al., 2009). This perhaps justifies the already locally established function of the plant in the treatment and management of hypertension (Duke, 1985). Alkaloids have been found to have microbiocidal effect and their antidiarrheal effect is probably due to their action on small intestine. In addition, they effect antihypertensive antifungal, anti-inflammatory, and anti fibrogenic effect (Awoyinka et al., 2007). However, the results of this work are similar to the findings of McDevitt et al. (1996) who reported the presence of alkaloid in *Cnidoscolusa conitifolius.* Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (Scalbert, 1991).

The results of quantitative analysis of the parts of *S. alata* as presented in Table 2 showed saponin contents of 40.57±0.57 and 33.02±0.07 mg/100 g for leaf and root bark respectively. It was observed that saponin concentrations were higher in leaf than root bark. These results were high compared to 12.1 mg/100 g of *M. utilis* reported by Siddhuraju and Becker (2005). Saponins are naturally occurring surface – active glycosides. They are mainly produced by plants, but also by lower marine animals and some bacteria (Riguera, 1997). The results of quantitative analysis of alkaloid content obtained from this plant organ were 14.09±0.50 and 15.89±0.72 mg/100 g for leaf and root bark respectively. The alkaloid contents were higher in root bark than leaf. This is similar to the values reported for *S. alata* flower (8.50±0.01 mg/100 g) by Abdulwaliyu et al. (2013). Alkaloids are more or less toxic substances which act primarily on the central nervous system (Hegnuauer, 1963). The tannin contents analyzed in this work were 59.48±0.50 and 44.38±0.72 mg/100 g for leaf and root bark respectively. The concentration was high with *S. alata* leaf while root bark had the least. The contents of tannin obtained in these were similar to 46.08 mg/100 g of *M. utilis* reported by Siddhuraju and Becker (2005). The values of flavonoids analyzed from the two samples were 42.28±0.90 and 36.52±0.38 mg/100 g for leaf and root bark respectively. The flavonoid contents were higher in *S. alata* leaf than root bark. The phenol contents analyzed from this work were 7.84±0.49 and 9.91±0.68 mg/100g for leaf and root bark respectively. The concentration of the root bark was found to be high while leaf had the least value. These values were high compared to 2.00±0.21 mg/100 g for *S. alata* leaf reported by Abdulwaliyu et al. (2013).

Anti-nutritional factors affect the availability of nutrients required by the body and interfere with metabolic process so that growth and development of the body is negatively influenced (Richard et al., 2006). The results of anti-nutritional factors obtained for this work were presented in Table 3. The phytate content in the samples analyzed was 12.44±0.31 and 15.07±0.58 mg/100 g for root bark and leaf respectively. The content of phytate was higher in *S. alata* leaf. Phytate helps in adequate iron bioavailability. The result obtained in this study was high when compared to the 3.55 mg/100 g of *S. alata* leaf reported by Abdulwaliyu et al. (2013). The contents of oxalates obtained from this work were 7.84±0.74 and

Table 3. Anti-nutrient factors of leaf and root bark of *S. alata* (mg/100 g).

Anti-nutrient constituents	Samples	
	Leaf	Root bark
Oxalate	7.84±0.74[b]	9.91±0.62[a]
Cyanide	21.69±0.11[a]	13.04±0.09[b]
Phytate	15.07±0.58a	12.44±0.31[b]

Values are means ±SD of three determinations, different superscripts along the same row are significantly different (p≤0.05).

Table 4. Determination of Antioxidant contents of leaf and root bark of *S. alata*.

Antioxidant contents	Samples	
	Leaf	Root bark
Phenolic properties (mg/g)	1.47±0.66[b]	3.72±0.35[a]
Ferric reducing properties (µmol/mg)	0.62±0.13[a]	0.32±0.24[b]

Values are means ±SD of three determinations, different superscripts along the same row are significantly different (p≤0.05).

Table 5. Chemical compounds deduced from GC-MS spectrum of *S. alata* leaf.

Line no	IUPAC Name	Molecular formula	Molar mass	RT	Area %
1	2-Methyl-1-Butanoic acid	$C_7H_{14}O_2$	130	7.275	4.56
2	2-Butylhydrazone	$C_4H_{10}N_2$	86	11.683	1.43
3	β-D-Glucopyranoside	$C_7H_{14}O_6$	194	13.525	12.59
4	Glycolaldehyde dimer	$C_4H_8O_4$	120	13.958	8.98
5	α-d-Mannofuranoside	$C_7H_{14}O_6$	194	15.058	53.35
6	Decanoic acid	$C_{12}H_{24}O_2$	200	16.367	0.27
7	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	16.617	2.7
8	11,14,17-Eicosatrienoic acid	$C_{21}H_{36}O_2$	320	17.525	0.77
9	2-Eicosene-1-ol	$C_{20}H_{40}O$	296	17.7	1.96
10	Oleic acid	$C_{18}H_{34}O_2$	282	18.425	12.3
11	3-Decen-1-ol	$C_{10}H_{20}O$	156	20.767	1.1

9.91±0.62 mg/100 g for leaf and root bark respectively. The concentration of oxalate was higher in *S. alata* root bark than the leaf. Similar values were obtained for the *S. alata* leaf (8.03±0.06 mg/100 g) reported by Abdulwaliyu et al. (2013). The presence of oxalate in food causes irritation in the mouth and interferes with absorption of divalent minerals particularly calcium by forming insoluble salts (Ola and Oboh, 2000). The cyanide content in the samples analyzed ranged from 13.04±0.09 and 21.69±0.11 mg/100 g for root bark and leaf respectively. The contents of cyanide were higher with *S. alata* leaf. These values are low when compared to the toxic level of 26.05±0.45 mg/100 g, reported for *S. alata* leaf by Abdulwaliyu et al. (2013).

Table 4 shows the antioxidants properties obtained from this study using phenolic and ferric reducing properties. The antioxidant values of leaf and root bark were 1.47±0.66 and 3.72±0.35 mg/g respectively for phenolic properties with root bark exhibiting higher value. These values were similar to 3.58 mg/g reported for *S. hirsute* by Essiett and Bassey (2013). The ferric reducing properties obtained from this work were 0.62±0.13 and 0.32±0.24 µmol/mg for leaf and root bark respectively. From these results, high ferric reducing properties was recorded for leaf (0.62±0.13 µmol/mg) over root bark (0.32±0.24 µmol/mg). These values were higher when compared to 0.17±0.04 µmol/mg reported for *Pueraria mirifica* by Buran and Supak (2007).

Tables 5 to 6 show the analytical parameters for GC-MS for the two organs of *S. alata*. It was observed that the organ of this plant contains all important fatty acid needed in the body for proper functioning. The fatty acids

Table 6. Chemical compounds deduced from GC-MS spectrum of *S. alata* root bark.

Line no	IUPAC Name	Molecular formula	Molar mass	RT	Area %
1	1,3-Dihydroxy-2-propanone	$C_3H_6O_3$	90	4.018	6.71
2	1,2,3-Propanetriol	$C_3H_8O_3$	92	5.199	21.54
3	α -D-Glucopyranoside	$C_7H_{14}O_6$	194	13.274	16.41
4	6-deoxy-L-Mannose	$C_6H_{12}O_5$	164	13.673	5.38
5	β-D-Mannofuranoside	$C_7H_{16}O$	194	14.615	22.41
6	n-Hexadecenoic acid	$C_{16}H_{32}O_2$	256	16.425	5.71
7	3-Methyl-1-hexanol	$C_7H_{16}O$	116	17.406	0.72
8	Oleic acid	$C_{18}H_{34}O_2$	282	18.104	14.65
9	Eicosanoic acid	$C_{20}H_{40}O_2$	312	18.258	1.89
10	6,11-Dimethyl-2,6,10-Dodecatrien-1-ol	$C_{14}H_{24}O$	208	20.393	3.43
11	1,10-Decanediol	$C_{10}H_{22}O_2$	174	20.709	1.14

recorded in this work are major source of energy. Most diets contain a great deal of fatty acids which were seen in this plant in form of triacylglycerol. The result of GC-MS spectrum of *S. alata* leaf, as presented in Table 5, revealed the presence of many major components, namely α-d-mannofuranoside, oleic acid, β-d-glucopyranoside, glycolaldehyde dimer and. The percentage concentration for each of the organic compounds was 53.35, 12.30, 12.59, 8.98 and 4.56% respectively (Table 5). The content of *S. alata* leaf was similar to *Cassia alata* reported by Isiaka et al. (2010).

The GC-MS spectra of *S. alata* root bark gave eleven compounds (Table 6). The major components were β-d-mannofuranoside, 1,2,3- propanetriol, α-d-glucopyranoside, oleic acid, 1,3-dihydroxy-2-propanone, n-hexadecenoic acid and 6-deoxy-L-mannose. The percentage concentration for each of the organic compounds were 22.41, 21.54, 16.41, 14.65, 6.71, 5.71 and 5.38% respectively. The contents of *S. alata* root bark were observed to be high when compared to *Senna podocarpa* used for medicinal purposes in Nigeria reported by Adebayo et al. (2014).

Conclusion

There is need for more research on the activity of the extracts in this plant against a wider range of bacteria and fungi and on the toxicology and further purification of the extracts for isolation of the pure active constituents. However, the two parts of the plant studied can contribute to human medication. It can be concluded that the plant contains various phytochemical constituents such as tannins, flavonoids, terpenoids, saponins, alkaloids, glycosides, steroids, phenol and anthraquinone. The presences of these secondary metabolites can inhibit the growth of micro-organisms and also have potentials of being developed for pharmaceuticals.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Abdulwaliyu I, Arekemase SO, Bala S, Ibraheem AS, Dakare AM, Sangodare R, Gero M (2013). Nutritional Properties of *Senna alata* Linn Leaf and Flower. Int. J. Mod. Biol. Med. 4(1):1-11.

Adams RP (2007). Identification of Essential Oil Components by Gas chromatography/Quadrupole Mass spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois, USA.

Adebayo MA, Lawal OA, Sikiru AA, Ogunwande IA, Avoseh ON (2014). Chemical Constituentsand Antimicrobial Activity of Essential Oil of *Senna podocarpa* (Guill. etPerr.) Lock. Am. J. Plt. scis. 5:2448-2453.

Awoyinka AO, Balogun IO, Ogunnowo AA (2007). Phytochemical screening and *in-vitro* bioactivity of *Cnidoscolus aconitiifolus* (Euphorbiaceae), J. Med. Pla. Res. 1(3):63-65.

Benzie IEF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 239:70-76.

Bell E (2007). Vitamin D3 promotes immune function on the skin. http//www.signalinggateway.org/upd ate/updates/

Buran P, Supak P (2007). Antioxidant Capacities of *Pueraria mirifica,Steviare baudiana* Bertoni, *Curcuma longa* Linn., *Andrographis paniculata*(Burm.f.) Nees.And *Cassia alata* Linn.for the Development of Dietary Supplement.Kase. J. Nat. Sci. 41:548–554.

Day RA, Underwood AL (1986). Qualitative Analysis. 5th Ed. New Delhi, India: Prentice Hall Publications. 701.

Duke JA (1985). Handbook of Medicinal Herbs .CRC press Inc. Boca Raton, Fla, pp. 72-80.

Edeoga HO, Okwu DE, Mbaebei BO (2005). Phytochemical constituents of some Nigerian Medicinal plants. Afr. J. Biot. 4(7):685-688.

Essiett UA, Bala DN, Agbakahi JA (2010). Pharmacognosticstudies of the leaves and stem of *Diodiascandens* SW in Nigeria. Archives of Appl. Sci. Res. 2(5):124-198.

Essiett UA, Bassey IE (2013). Comparative Phytochemical Screening and Nutritional Potentials of the Flowers (petals) of *Senna alata* (L) roxb, *Senna hirsute* (L) Irwin and barneby, and *Senna obtusifolia* (L.)Irwin and barneby (Fabaceae). J. Appl. Pharm. Sci. 3(08):097-101.

Evans WC (2002). Pharmacognosy, (15th edition), London W.B. Saunders Company Ltd., pp. 191-393.

Harborne JB (1989). Biosynthensis and function of antinutritional factors in plants. Aspects Appl. Biol. 19:21-25.

Hegnuauer R (1963). Chemical Plant Taxonomy. Academic Press Inc.,

New York, P. 389.
Isiaka AO, Guido F, Pier LC, Omikorede O, Ridwan AA, Akinwunmi AA, Yusuff OK (2010). Aromatic plants growing in Nigeria: Essential oil constituents of *Cassia alata* (Linn.) Roxb. and *Helianthus annuus* L. Record of Natural Products. 4(4):211-217.
Koleva II, Niederlander HAG, Van Beek TA (2000). An online HPLC method for detection of radical scavenging compounds in complex mixtures. Anal. Chem. 72:2323-2328.
Maynard LA (1997). Animal nutrition. McGraw Hill book company Ltd. New York, P. 47.
McDevitt JT, Schneider DM, Katiyar SK, Edlind FS (1996). Berberina: a candidate for the treatment of diarrhea in AIDS patients abstract 175, In program and Abstracts of the 36th Interscience on ference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Washington, D. C.
Midawa SM, Ali BD, Mshelia BZ, Johnson J (2010). Cutaneous wound healing activity of the ethanolic extracts of the leaf of *Senna alata* L. J. Biol. Sci. Bio. 26(5):343–356.
Ola FL, Oboh G (2000). Anti-nutritional factors, in nutritional quality of plant foods. J. Technol. 4:1-3.
Onwuka GI (2005). Food analysis and instrumentation theory and practice. Naphthali print, Nigeria. pp. 63-98.
Orishadipe AT, Okogun JI, Mishelia E (2010). Gas chromatography - mass spectrometry analysis of the hexane extract of *Calliandra portoricensis* and its antimicrobial activity. Afr. J. Pure Appl. Chem. 4(7):131-134.
Richard W, Katie E, Ferrell T (2006). Squirrels: the animal answer guide. Baltimore: Johns Hopkins University Press. seeds. Pakistan J. Nut. 6:40-43.
Riguera R (1997). Isolating bioactive compounds from marine organisms. J. Mar. Biotech. 5:187-193.
Schuler P (1990). Natural antioxidants exploited commercially. Food Antioxidants, Elsevier Appl. Food Sci. Ser. pp. 99-170.
Scalbert A (1991). Antimicrobial properties of tannis. Phytochemistry 30:3875-3882.
Siddhuraju P, Becker K (2005). Nutritional and antinutritional composition, *in vitro* amino acid availability, starch digestibility and predicted glycemic index of differently processed mucuna beans (*Mucuna pruriens* var. utilis): An underutilized legume. Food Chem. 91:275-286.
Stein S, Mirokhin D, Tchekhovskoi D, Mallard G (2002). The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectra Library. Standard Reference Data Program of the National Institute of Standards and Technology. Gaithers-burg, MD, US; 2002.
Sparg SG, Light ME, Stadan JV (2004). Biological activities and distribution of plant saponins. J. Ethnoph. 94:219-243.
Taiwo A, Abidemi C, Oyedepo J, Adebayo B, Oluwadare I, Agboto D (2009). Nutrient content and antinutritional factor in Shea butter (*Butryospermum parkii*) leaves. Afr. J. Biotech. 8(21):5888-5890.
Trease MT Evans SE (1989).The phytochemical analysis and anti-bacterial screening of extract of some common vegetables. Chem. Sci. Nig. 26:57-58.
Valentao P, Fernandes E, Carvalho F, Andrade PB, Seabra, RM. Bastos ML (2002). Antioxidant properties of cardoon (*Cynara cardunculus* L.) infusion against superoxide radical, hydroxyl radical, and hypochlorous acid. J. Agric. Food Chem. 50:4989-4993.
Veerachari U, Bopaiah AK (2011). Preliminary phytochemical evaluation of the leaf extract of five *Cassia* species. J. Chem. Pharm. Res. 3(5):574-583.
Wheeler EI, Ferrel RE (1971). Methods for phytic acid determination in wheat and wheat fractions. J. Cer. Chem. 48:312-320.
World Health Organization (2002). General Guidelines for methodologies on research and evaluation of Traditional medicines. Geneva, World Health Organization. 8:235-239.

academicJournals

Vol. 9(5), pp. 98-104, May, 2015
DOI: 10.5897/AJPAC2015.0616
Article Number: 569FBFA52896
ISSN 1996 - 0840
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJPAC

African Journal of Pure and Applied Chemistry

Full Length Research Paper

Evaluation of anti-oxidant properties in essential oil and solvent extracts from *Tagetes minuta*

Kyarimpa Christine[1]*, Isaiah Ndiege Omolo[1], John David Kabasa[2], Christine Betty Nagawa[3], John Wasswa[4] and Cliff Richard Kikawa[5]

[1] Department of Chemistry, Faculty of science, Kyambogo University Uganda.
[2]College of Veterinary medicine, Makerere University Uganda
[3]Department of Forestry, Biodiversity and Tourism School of Forestry, Environment and Geographical Sciences College of Agricultural and evironmental Sciences, Makerere University Uganda.
[4]Department of Chemistry, College of Natural Sciences, Makerere University, Uganda.
[5]Tshwane University of Technology(SA), Faculty of Science, Dept of Mathematics and Statistics. Arcadia Campus- Pretoria.

Received 9 February, 2015; Accepted 27 April, 2015

Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are toxic and carcinogenic, thus they induce DNA damage. This calls for the need to find alternative antioxidants from natural products. *Tagetes minuta* (Asteraceae) is an annual herb that belongs to the Asteraceae family. It is used in common medicine and grows in temperate regions of South America, some parts of Africa and Asia. Essential oil from *T. minuta* was obtained by hydrodistillation while solvent extracts were obtained using ethyl acetate and methanol. Antioxidative compounds of *T. minuta* were isolated both from the Ethyl acetate extract and the essential oil. It was done by determining the scavenging activity using 2,2-diphenyl-1-picrylhaydrazyl free radical (DPPH) using thin layer chromatography (TLC). The active compounds were tested quantitatively for their radical scavenging activity using the U-1100 UV-VIS Spectrophotometer. The active fractions were isolated using TLC and High performance liquid chromatography (HPLC) and later detected using both Gas chromatography mass spectrometry (GC-MS) and Nuclear magnetic resonance (NMR). One pure active compound was obtained from the ethylacetate extract (neophytadene) by a combintion of GC-MS and NMR. The essential oil contained a number of compounds among which are trans-ocimen 15.90%, I-verbanone 15% of limonene 8.02%, tegetone 3.56%, and 2-pinen-4-one 7.84%.

Key words: Antioxidant activity, essential oil, 2,2-diphenyl-1-picrylhaydrazyl free radical (DPPH), *Tagetes minuta*.

INTRODUCTION

An antioxidant is any substance, if present at low concentrations in combination with an oxidisable substrate, significantly delays or prevents oxidation of the substrate. Based on the historical success of natural products, a number of medicinal plants have been evaluated for their antioxidant potential (Argolo et al., 2004; Burits et al., 2001; Helle et al., 2004).

Free radical mediated damage is connected with

*Corresponding author. E-mail: xtineky@gmail.com, Tel: +256772561171.
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

several diseases, and therefore its prevention can play an important role in the cure of those diseases (Kanwal et al., 2011; Kulisica et al., 2004; Sharma and Trivedi, 2002; Smith et al., 2007). For example, oxidative stress has been widely postulated to be involved in the development and progression of some chronic diseases such as cardiovascular disease, neuronal disease, cataracts, and several types of cancer (Gua et al., 2009). There is increasing search for antioxidants that remove occurring naturally in vegetables, fruits and functional herbs to replace synthetic antioxidants. It has been found out that some synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been revealed to be potentially toxic and carcinogenic, they have been found to induce DNA damage (Helle et al., 2004).

Tagetes minuta (Asteraceae) is an annual herb that belongs to the Asteraceae family. Its leaves are slightly glossy, green and pinnately dissected into 4–6 pairs of pinnae (Cerruti et al., 2010; Daizy et al., 2007). It is used in common medicine and grows in temperate regions of South America, some parts of Africa and Asia (Hamill et al., 2000; Kamatenesi-Mugisha and Oryem-Origa, 2007; Tabuti et al., 2003; Vasudevan et al., 1997a). Infusions of leaves from different species of *Tagetes* have been used to treat stomach and intestinal diseases (Gakuya et al., 2013; Harris et al., 2002; Paul and Kasenene, 2007; Tabuti et al., 2003; Vasudevan et al., 1997a), and other species have been found to possess different biological activities, such as, antimicrobial, antiinflammatory, antioxidant and antiviral (Andreotti et al., 2013; Dharmagadda et al., 2005; Hamil et al., 2000; Paul and Kasenene, 2007).

T. minuta is a wild shrub in Uganda that thrives mostly in the rainy season (Tabuti et al., 2003). Tagetes species, commonly known as marigold are also grown as ornamental plants and thrive in varied agro-climates (Vasudevan et al., 1997b). Bioactive extracts of different Tagetes parts exhibit nematocidal, fungicidal and insecticidal activity (Vasudevan et al., 1997b). *T. minuta* has been used by the local people in Uganda to relieve a number of ailments (Hamil et al., 2000; Paul and Kasenene, 2007).

Previous work on *Tagetes* species, *Tagetes maxima* reavealed strong antioxidant properties of its ethylacetate extracts (Parejo et al., 2005). *T. maxima* was found to exhibit strong radical scavanging and antioxidant activities (Parejo et al., 2005) .There is a great possibility of similar activity in other *Tagetes* species. Antioxidant activity of *T. minuta* from Uganda has not been determined according to literature, but since it belongs to the same family as other Tagetes species with strong antioxidant properties, it was necessary to determine its potential as an antioxidant.

In this research, antioxidative compounds of *T. minuta* were isolated both from the Ethyl acetate extract and the essential oil. It was done by determining the scavenging activity using 2,2-diphenyl-1-picrylhaydrazyl free radical (DPPH). The active compounds were tested quantitatively for their radical scavenging activity.

EXPERIMENTAL

Plant material

Fresh aerial parts of *T. minuta* were collected from Mabira Forest in the morning hours in the month of November 2012. The sample was transported to Makerere University, Department of Chemistry Laboratory. Essential oils from *T. minuta* were extracted on arrival in the Laboratory. The remaining plant material was dried under shade for 3 weeks, ground in a mortal to obtain fine powder. A voucher specimen (CK001) was deposited at Makerere University Herbarium.

Hydrodistillation

Essential oil from fresh *T. minuta* was extracted by hydro-distillation in a Clevenger type apparatus for 3 h with a separated extraction chamber. The resulting essential oils were dried over anhydrous-sodium sulphate to extract the water. The oil was kept in refrigerated conditions at 8°C prior to the antioxidant activity determination and GC-MS analysis (Conti et al., 2010; Polatoglu et al., 2012).

Chemicals

All chemicals and reagents used in extraction, isolation and analysis of the active compounds were obtained from Sigma-Aldrich (Germany). These chemicals and reagents were of analytical grade. The standards were also purchased from sigma-Aldrich.

Solvent extraction- Cold extraction

T. minuta dry powder (1000 g) was extracted four times with 2000 ml of ethyl acetate at 40 to 45°C. The supernatant (extract) was separated from the residue by paper filtration (Whatman No. 1 filter, whatman paper Ltd., UK). It was dried in vaccum using a rotary evaporator at 40°C to remove all the ethyl acetate to give a residue. The powder was dried and re-extracted three times with 2000 ml methanol. The extract was combined and evaporated at 40°C to dryness. Both methanolic and ethyl acetate extracts were kept in a dry place for further testing (Gua et al., 2009).

DPPH assay

The capacities to donate hydrogen atoms/electrons by the essential oil and solvent extracts from the test samples were preliminarily detected using thin layer chromatography (TLC) and further measured spectrophotometrically.

TLC screening for antioxidants

Dilutions of volatile oils (5 µl, 1:5 in hexane), and the crude extracts were spotted on silica gel sheets and developed in ethylacetate:hexane (7:3 v/v). The plates were sprayed with 0.2% solution of the stable radical, diphenylpicrylhydrazil (DPPH) (Brand Williams et al., 1995; Burits et al., 2001; Helle et al., 2004; Xiao et al., 2010). Active spots were detected as yellow spots on a purple

background. Zones where the colour changed within 30 min (after spraying) were taken as positive results (Burits et al., 2001).

DPPH spectrophotometric assay

This assay uses DPPH as a reagent (Argolo et al., 2004; Brand-Williams et al., 1995; Burits et al., 2001; Helle et al., 2004). 50 µl of various concentrations of the volatile oils were added to 5 ml of 0.004% methanolic solution of DPPH. After 30 min of incubation period at room temperature, the absorbance was read against the blank at 517 nm using a U-1100 UV-VIS Spectrophotometer (Hitachi Ltd; Tokyo Japan). The tests were carried out in duplicate. DPPH solution (1.0 ml; 0.3 mM) plus methanol (2.5 ml) was used as a negative control. After 30 min the absorbance values were measured at 517 nm and converted into the percentage antioxidant activity (AA) using the following formula:

%AA, which was, $\%aa=((A_{C(0)} - A_{A(t)})/A_{C(0)})*100$

Where $A_{C(0)}$ is the AA for the control solution at t=o minutes, and $A_{A(ti)}$ is the AA after the given time intervals, for I = 5, 10, 15, 20, 25, and 30 min (Kulisica et al., 2004).

GC-MS analysis

GC-MS analysis was used to identify the compounds in the essential oil and solvent extracts that had antioxidant activity.The GC-MS results of *T. minuta* was already determined in a previous research (Kyarimpa et al., 2014).

High performance liquid chromatography

The active fractions were purified with HPLC. A Dionex Ultimate 3000 HPLC (Dionex) equipped with a diode array detector and operated by Chromeleon Version 6.80 SR9 software. 2.0 ml each of the active fractions was injected onto a 150 x 2.1 mm, 100 A, 2.6 µm Phenomenex Kinetex C18-column at 35°C. N-Hexane was used as a mobile phase. The flow rate was 237 µl/min. To selectively detect antioxidants, the detector recorded the signal at 520 nm (Application Note 281, Dionex Corporation, Sunnyvale, CA, USA). HPLC was carried out only samples with antioxidant activity and the solvent system was chosen based on Rf values of the TLC experiments.

Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is (arguably) the most powerful tool available for determining the structure of organic compounds. It is used to identify and/or elucidate detailed structural information about chemical compounds. In this case it was used to determine the structure of the pure active compound in the sample. All NMR spectra were recorded on a Bruker Avance II 400 (resonance frequencies 400.13 MHz for ^{1}H and 100.63 MHz for 13C) equipped with a 5 mm broadband observe probe head (BBFO) with z–gradients at room temperature with standard Bruker pulse programmes. The sample was dissolved in 0.6 ml of CDCl3 (99.8% D). Chemical shifts are given in ppm, referenced to residual solvent signals (7.26 ppm for ^{1}H, 77.0 ppm for ^{13}C). ^{1}H NMR data were collected with 32k complex data points and apodized with a Gaussian window function (lb = −0.3 Hz and gb = 0.3 Hz) prior to Fourier transformation. ^{13}C-jmod spectra with WALTZ16 ^{1}H decoupling was acquired using 64k data points. Signal-to-noise enhancement was achieved by multiplication of the FID with an exponential window function (lb = 1Hz). All two-dimensional experiments were performed with 1k × 256 data points, while the number of transients (2–8 scans) and the sweep widths were optimized individually. The resulting FIDs were zero-filled to a 2k × 1k data matrix and apodized with a sine function for COSY in both the ω1 and ω2 dimensions prior to Fourier transformation.

Heteronuclear spectra were zero-filled only in F1 to a 1k × 512 data matrix, and apodized in both dimensions with a shifted sine function. The heteronuclear single quantum coherence (HSQC) experiment was acquired using adiabatic pulse for inversion of ^{13}C and GARP-sequence for broadband ^{13}C-decoupling, optimized for 1J(CH) = 145 Hz.

RESULTS AND DISCUSSION

The essential oil from tagets minuta exhibited strong antioxidant activity on TLC. Some components of the crude extract were also found to be active (Figure 2). Track 1 on the first TLC plate from the left (Figure 2) was the essential oil, the other tracks were different extracts from *T. minuta* and *Tephrosia Vogelli*. It was noted that some of the components of the two plants had strong antioxidant activity. These fractions were isolated using Column Chromatography, HPLC. The pure fractions were analysed using NMR spectroscopy. Track 1 had very strong antioxidant activity as shown by the DPPH reaction on the TLC plate (Figure 2). This oil was later used for quantitative measurement again using DPPH and Ultra Violet Spectrophotometer (Table 1). The total antioxidant capacity revealed that the essential oil from *T. minuta* had a high antioxidant activity. Free radical scavenging activity of the extracts was assesed using the stable free radical DPPH. Plant extracts which reduce DPPH by donating hydrogen ions are considered as antioxidants having free radical scavenging activity. The results from Table 1, were used to calculate the amount of DPPH scavenged over a period of time according to the formula {%AA, which was , %aa=((AC(0) – AA(t))/AC(0))*100}, and the LC_{50} was determined (Appendix 1B). DPPH solution alone served as control (A0). It is evident from the study, that the investigated extracts and essential oil have the ability to quench free radicals. This indicates that *T. minuta* is a potential source of natural antioxidants.

Nuclear magnetic resonance (NMR) Spectroscopy

One active pure compound was identified using both the proton NMR and the carbon NMR as shown in Figure 1 and Table 2. The 1 H NMR spectra of this compound revealed the presence of two isolated olefinic spin systems without any further coupling partners: on the one hand a vinyl group, indicated by its characteristic ABX-system at α 5.05 (d, J = 10.8 Hz), α 5.23 (d, J = 17.6 Hz) and α 6.37 (dd, J = 17.6 , 10.8 Hz), and on the other hand an olefinic methylene group with broad singuletts at α 4.99 and α 5.00 ppm, respectively. Besides a triplett at

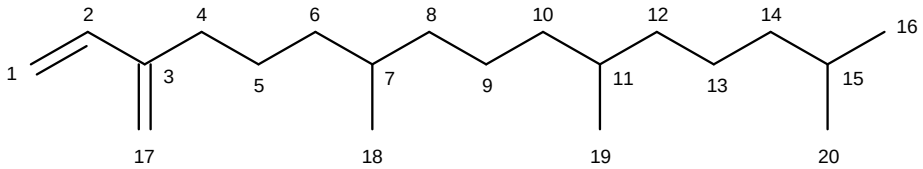


Figure 1. The structure of Neophytadiene

Table 1. Ultraviolet Spectroscopic results showing the effect of sample concentration on the absorbance of DPPH.

Conc. (g/L)	Absorbance (min)						
	0 (Control)	5	10	15	20	25	30
DPPH(Blank)	1.063	1.037	1.016	0.995	0.975	0.954	0.935
5	0.225	0.151	0.11	0.08	0.057	0.05	0.049
5	0.223	0.14	0.09	0.055	0.046	0.046	0.045
10	0.0132	0.046	0.034	0.034	0.034	0.035	0.035
10	0.191	0.086	0.045	0.045	0.045	0.045	0.045
20	0.179	0.066	0.056	0.055	0.055	0.055	0.055
20	0.206	0.077	0.059	0.058	0.059	0.059	0.06
30	0.134	0.069	0.076	0.081	0.085	0.086	0.085
30	0.156	0.082	0.074	0.064	0.053	0.042	0.032
40	0.16	0.06	0.063	0.066	0.069	0.07	0.072
40	0.166	0.057	0.063	0.063	0.065	0.064	0.062
60	0.118	0.051	0.053	0.053	0.049	0.041	0.032

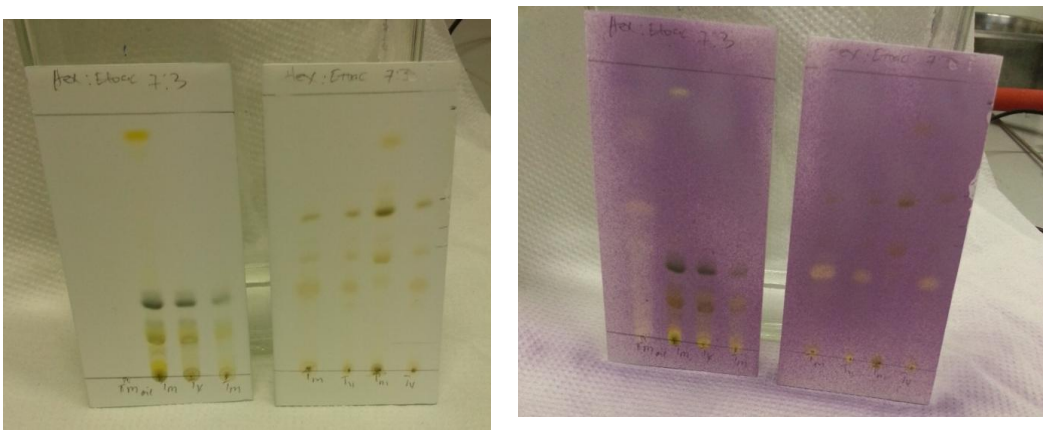


Figure 2. TLC plate before (left) and after applying DPPH (right).The yellow spots on a purple background indicate antioxidant activity of the compounds at that particular spot.

α 2.18 ppm, a bulk of aliphatic methylene and methine protons in the region between 1.60 to 1.00 ppm and In combination with the hsqc experiment the j-modulated 13C nmr spectra showed signals of 4 olefinic carbons – one quaternary, one methine, and two methylenes – 4 methyl, 3 methine and 9 methylene carbons. In addition to the molecular mass peak at m/z = 278 these results indicated that compound is a noncyclic, nonoxidized diterpene with a molecular formula of $C_{20}H_{38}$. Extensive signals of different methyl groups at around 0.90 ppm no more signals were found in the 1H NMR spectra. analysis of heteronuclear 2D NMR led to the elucidation of that structure which turned out to be neophytadiene, a widespread component of essential oils from different plant sources. Whereas Burkhardt et al. published only NMR data of the olefinic part of the molecule, we present here to our best knowledge for the first time the fully assigned nmr resonances (Table 2).

Table 2. NMR resonance of the fully assigned Neophytadiene.

	1H	13C
1[a]	5.23, d, J = 17.6	113.02
1[b]	5.05, br.d, J = 10.8	
2	6.37, dd, J = 17.6 + 10.8	139.07
3	-	146.69
4	2.18, m	31.70
5	1.49, m	25.63
6	1.00 - 1.30	36.99
7, 11	1.38, m	32.80 + 32.69
8, 10, 12	1.00 - 1.30	37.44 + 37.39 + 37.30
9, 13	1.00 - 1.30	24.80 + 24.46
14	1.15, m	39.38
15	1.53, m	27.99
16, 20	0.87	22.72 + 22.62
17	5.00, br.s	115.42
	4.99, br.s	
18, 19	0.85	19.75 + 19.74

Neophytadiene, is a fatty acid-related compound which plays an important part in competitive inhibition of cyclooxygenase or lipoxygenase in an inflammation reduction, resulting in decreased production of prostaglandins and leukotriene (Pillai and Nair, 2013).

Conclusion

DPPH is a free radical, stable at room temperature, which produces a violet solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol solutions. The use of DPPH provides an easy and rapid way to evaluate antioxidants. According to the results obtained from data in Tables 1 and 2 as well as those of statistical analysis, we can say that extracts from *T. minuta* as well as its essential oil possess strong antioxidant properties with an LC_{50} of 1.49 g/l^{-1} after 30 min as compared to other antioxidants reported in Parejo et al. (2005) and Xiao et al. (2010). A well-known antioxidant, ascorbic acid, was used as positive control. DPPH scavenging patterns for T. minuta versus time, along with IC50 values, are presented in Appendix 1 A and B.

Conflict of Interest

We declare no competing interests in this research.

ACKNOWLEDGEMENT

The authors wish to acknowledge the Robert Mc. Namara Fellowship Scheme of the World Bank for the sponsorship. The University of Natural Resources and Applied life Sciences, Vienna for hosting the researcher and providing techinical guidance. Special thanks go to Prof. Stefan Bohmdorfer ,Prof Thomas Rosenau and Dr. Markus Bacher for their techinical support. The authors express their gratitude to CARNEGIE Corporation of New York for the financial support through The Science Initative Group's RISE-AFNNET, Makerere University.

REFERENCES

Andreotti R, Garcia MV, Cunha RC, Barros JC (2013). Protective action of *Tagetes minuta* (Asteracea) essential oil in the control of *Rhipicephalus microplus* (Canestrini, 1887) (Acari: Ixodidae) in a cattle pen trial. Veterinary parasitol. 197:341-345.

Argolo ACC, Sant'Ana AEG, Pletsch M, Coelho LCBB (2004). Antioxidant activity of leaf extracts from Bauhinia monandra. Bioresour. Technol. 95:229-233.

Brand-Williams W, Cuvelier ME, Berset C (1995). Use of fre radical method to evaluate Antioxidant Activity. Lebensm. Wiss. U-Technol. 28:25-30.

Burits M, Asres K, Bucar F (2001). The antioxidant activity of the essential oils of Artemisia afra, *Artemisia abyssinica* and *Juniperus Procera*. Phyotherapy Res. 15:103-108.

Cerruti RRH, Koon-Hui W, Antoon P, Robert M (2010). Using marigold (Tagetes spp.) as a cover crop to protect crops from plant-parasitic nematodes. Appl. Soil Ecol. 46:307-320.

Conti B, Canale A, Bertoli A, Gozzini F, Pistelli L (2010). Essential oil composition and larvicidal activity of six Mediterranean aromatic plants against the mosquito *Aedes albopictus* (Diptera: Culicidae). Parasitol. Res. 107:1455-1461.

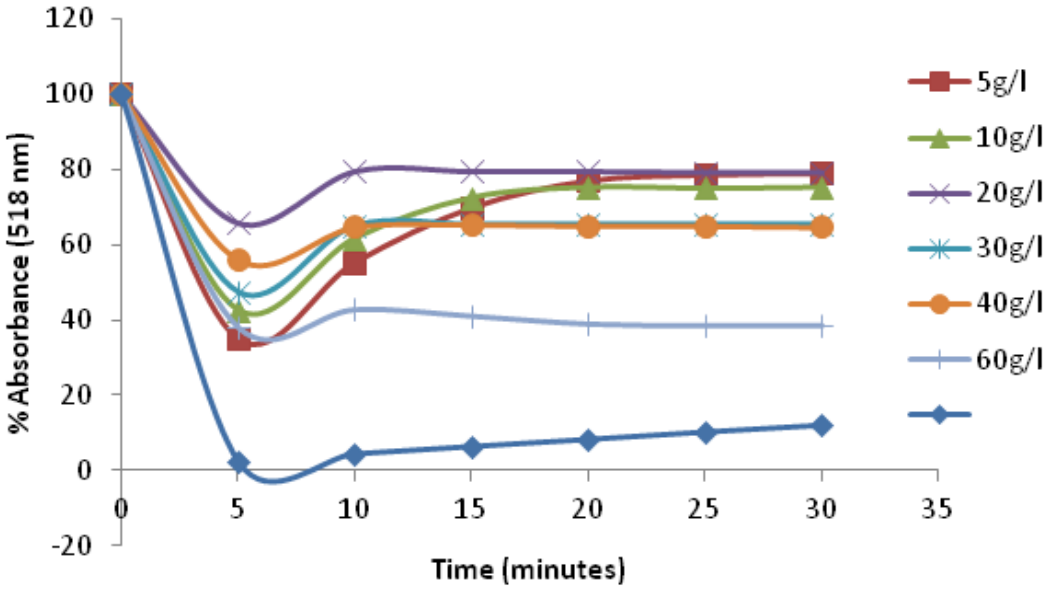
Daizy RB, Arora K, Singh HP, Kohli RK (2007). Potential utilization of dried powder of *Tagetes minuta* as a natural herbicide for managing rice weeds. Crop Protection. 26:566-571.

Dharmagadda VSS, Naik SN, Mittal PK, Vasudevan P (2005). Larvicidal activity of Tagetes patula essential oil against three mosquito species. Bioresour. Technol. 96:1235-1240.

Gakuya DW, Itonga SM, Mbaria JM, Muthee JK, Musau JK (2013). Ethnobotanical survey of biopesticides and other medicinal plants traditionally used in meru central district of Kenya. J. Ethnophamacol. 145:547-553.

Gua L, Wua T, Wanga Z (2009). TLC bioautography-guided isolation of antioxidants from fruit of Perilla frutescens var. Acuta LWT - Food Sci. Technol. 42:131-136.
Hamil FA, Apio S, Mubiru NK, Mosango M, Bukenya-Ziraba R, Maganyi OW, Soejarto DD (2000). Traditional Herbal drugs of southern Uganda. J. Ethnophamacol. 70:281-300.
Helle W, Anne SB, Malterud KE (2004). Antioxidant activity in extracts from coriander. Food Chem. 88:293-297.
Kamatenesi-Mugisha M, Oryem-Origa H (2007). Medicinal plants used to induce labour during childbirth in western Uganda. J. Ethnophamacol. 109:1-9.
Kulisica T, Radonic A, Katalinic V, Milosa M (2004). Analytical, Nutritional and Clinical Methods Use of different methods for testing antioxidative activity of oregano essential oil. Food Chem. 85:633-640.
Kyarimpa CM, Böhmdorfer S, Wasswa J, Kiremire BT, Ndiege IO, Kabasa JD (2014). Essential oil and composition of *Tagetes minuta* from Uganda. Larvicidal activity on Anopheles gambiae. Industrial Crops Prod. 62:400-404.
Harris PWG, Prince SA, Olsen RJA, Oketch-Rabah RAOH, Madiega PA, Andersen A, Molgaard P (2002). Medicinal plants used by Luo mothers and Children in Bondo district, Kenya. J. Ethnophamacol. 83:39-54.
Parejo I, Bastida J, Viladomat F, Codina C (2005). Acylated quercetagetin glycosides with antioxidant activity from *Tagetes maxima*. Phytochemisty 66:2356-2362.
Paul S, Kasenene JM (2007). Medicinal plant diversity and uses in the Sango bay area, Southern Uganda. J. Ethnophamacol. 113:521-540.
Pillai LS, Nair BR (2013). GC-MS Analysis of Chloroform Extract of Cleome Burmanni W. and A. (Cleomaceae). Int. J. Phamaceutical Sci. Res. P. 4.
Polatoglu K, Demirci B, Demirci F, Goren N, Baser KHC (2012). Biological activity and essential oil composition of two new Tanacetum chiliophyllum (Fisch. & Mey.) Schultz Bip. Var. Chiliophyllum chemotypes from Turkey. Industrial crops and products 39:97-105.
Tabuti JRS, Lye KA, Dhillion SS (2003). Traditional herbal drugs of Bulamogi, Uganda: Plants, use and administration. J. Ethnopharmacol. 88:19-44.
Vasudevan P, Kashyap S, Sharma S (1997a). TAGETES: A multipurpose plant. Bioresour. Technol. 62:29-35.
Vasudevan P, Kashyap S, Sharma S (1997b). Tagetes: A multipurpose plant. Bioresource Technol. 62:29-35.
Xiao G, Li G, Chen L, Zhang Z, Yin JJ, Wu T, Cheng Z, Wei F, Wang Z (2010). Isolation of antioxidants from Psoralea corylifolia fruits using high speed counter-current chromatography guided by thin layer chromatography-antioxidant autographic assay. J. Chromatography A. 1217:5470-5476.

APPENDIX A: Percentage absorbance of DPPH at different concentrations of the Essential oil of *Tagetes* Minuta vs time in minutes



We notice that radical scavenging or absorbance is inversely proportional to time.

APPENDIX B: LC_{50} Values at Different time Intervals

SN	Duration (Minutes)	LC_{50} gl^{-1}
	5	3.08
	10	1.87
	15	1.65
	20	1.55
	25	1.54
	30	1.49

academicJournals

Vol. 9(5), pp. 105-112, May, 2015
DOI: 10.5897/AJPAC2015.0620
Article Number: 79274D952898
ISSN 1996 - 0840
Copyright © 2015
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJPAC

African Journal of Pure and Applied Chemistry

Full Length Research Paper

Accessing the potential of *Lonchocarpus laxiflorus* roots (LLR) plant biomass to remove Cadmium (II) ions from aqueous solutions: Equilibrium and kinetic studies

A. H. Santuraki[1]* and A. A. Muazu

Department of Chemical Sciences, Federal University Kashere, Gombe State – Nigeria.

Received 22 March, 2015; Accepted 17 April, 2015

The potential of *Lonchocarpus laxiflorus* roots (LLR) for the removal of Cd (II) ions from aqueous solutions has been investigated. The adsorption of Cd (II) ions was found to be affected by solution pH, contact time, adsorbent dosage, initial metal ion concentration, and temperature. The equilibrium was analysed using Langmuir and Freundlich isotherm models. The data was found to have a closer correlation with the Lamgmur isotherm as evidenced by a higher correlation coefficient (R^2). The adsorption capacity for *L. laxiflorus* was found to be 19.35 mg g^{-1} and the removal efficiency of the LLR was found to be 98% under optimized conditions. The kinetics data was also subjected to pseudo-first-order and the pseudo-second-order kinetic models. The data could be explained better using the pseudo-second-order kinetic model. The selected biosorbent has been analyzed by FT-IR for identification of contributing functional groups, x-ray powder diffraction (XRD) for its structural properties and scanning electron microscope (SEM) for the structural morphology analysis.

Key words: Biosorption, cadmium, isotherms, kinetics, *Lonchocarpus laxiflorus*.

INTRODUCTION

Despite the requirement for healthy environment, the environment has been found to be contaminated with various pollutants. This has now posed a great challenge to human wellbeing. Such pollutants may be found in air, water soil, coastal erosion, overfishing and deforestation as well as disposal of waste, which constitute several heavy metals. Contamination of water by heavy metals is one of the most challenging environmental issues currently. Cadmium is one of the most toxic metals apart from lead and mercury. It has been reported to cause renal dysfunction, hypertension, lung insufficiency, bone lesions and cancer (Feng et al., 2010) which is a leading cause of death. The cadmium drinking water guidelines value recommended by WHO is 0.003 mg L^{-1} (WHO, 2008). Cadmium accumulates both in the environment and the body causing long term damage to life (Nida et al., 2012). Cadmium is one of the heavy metals with a greatest potential hazard to humans and environment

*Corresponding author. E- mail: santurakiah65@gmail.com
Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

(Fouad et al., 2012).

The principal sources of Cd into the environment are electroplating, smelting, alloy manufacturing, pigments, plastic, battery, mining and refining processes (Gupta and Nyaka, 2012). Once released into the environment cadmium is toxic to plants animals and microorganisms (Bailey et al., 1999). The metal is non-biodegradable, persistent and bioaccumulate mainly in the kidneys and liver of vertebrates, invertebrates and also in algae (Ajay et al., 2005). A number of methods have been employed to remove Cd (II) ions from the environment including, ion exchange, reverse osmosis (Gupta and Nyaka, 2012) membrane filtration, electrochemical treatment, and adsorption. etc where each of them have limitations such as high cost and production of hazardous by- products are found expensive and sometimes ineffective, especially when metals are present in solution at very low concentration within the range 1 to 100 mg/cm^3 (Santhi and Manonmani, 2012).

Consequently, it is essential to find new methods for effective removal of cadmium from water and wastewater. Compared with other traditional methods, adsorption is quite popular due to its simplicity and high efficiency, as well as the availability of a wide range of adsorbents (Orhan and Buyukgungor, 1993; Babel and Kurniawan, 2003). Activated carbon is the best useful adsorbent of heavy metals from waste water. However, the cost of activated carbon is high; its regeneration also requires additional expense. Therefore there is need to come up with other alternative that provide an easy, feasible, reliable, low cost adsorbents especially those of biological origin commonly referred to as biosorbents to improve the water quality. Biosorption has gained a lot of credibility currently because of its eco- friendly nature, excellent performance and cost- effectiveness (Davis et al., 2003). Equilibrium isotherm models and kinetic models were applied to the data obtained for a better understanding of the adsorption process. Thus the objective of the present study is to investigate the binding of metal ion cadmium (II) by *Lonchocarpus laxiflorus* roots (LLR) in its immobilized form from aqueous solutions and to study the effect of various factors affecting the efficiency of the process.

MATERIALS AND METHODS

Biomass preparation

Plant collection and treatment

The roots of *L. laxiflorus* plant were collected from a tree behind Modibbo Adama Federal University of Technology Yola, Nigeria. The plant was wash thoroughly under running water to remove dust and any adhering particle and then rise with distilled water. The sample was air dry for 2 weeks and the dry roots was grinded in analytical mill and sieve to obtain adsorbent of known particle size range. The biomass powder was kept in an air tight bottle for further study (Igwe and Abia, 2006). Our studies indicated that though there was no big difference in the adsorption rates of the various parts of plant, the roots were better. Therefore roots were used in this adsorption experiments.

Chemicals

All chemicals used in the present work were of analytical grade. The stock solution of Cd^{2+} ions was prepared in 1.0 g L^{-1} concentration. Cadmium solution of 1000 mg/cm^3 concentration was prepared by dissolving 2.103 g of $Cd(NO_3)_2$ in 250 cm^3 of distilled water and make up to 1000 cm^3 in a volumetric flask. The pH of the solutions was adjusted using 0.1 mol L^{-1} HCl and NaOH solutions.

Analysis of metal ions

Atomic absorption spectrophotometer equipped with an air acetylene flame, controlled with computer was used to investigate the concentration of cadmium metal ion. The hallow cathode lamp of Cd, was used at 283.3 nm wavelength, while slit is 0.2 nm, operated at 8 mA (Suleiman et al., 2007).

Reparation of sodium alginate and calcium chloride stock solution

Sodium alginate was prepared by weighing 4.00 g and making it up to 100 cm^3 mark with distilled water in a volumetric flask and left overnight for complete dissolution. 0.12 M of calcium chloride was prepared by weighing 26.28 g in to 1000 cm^3 volumetric flask and making up to mark with distilled water according to a standard procedure described by Osemeahon and Esenowo, (2012).

Immobilization of the roots of *L. laxiflorus* plant

Sodium alginate was used for immobilization of the roots of *L. laxiflorus* plant. 50 cm^3 of sample solution prepared by dissolving 4 g of each test sample in 100 cm^3 of distilled water and mix with 50 cm^3 of 4% stock solution of sodium alginate and stir vigorously in 250 cm^3 beaker, to obtain a homogenous mixture. After mixing, the solution the solution was drawn through hyperdemic needles and was added drop wise to a stirred solution of 1 M $CaCl_2$. A retention time of 1 h was allowed for the reaction to obtain complete precipitation of the immobilized leave powder of *L. laxiflorus* plant. The beads thus formed that is, sodium alginates were kept in fresh $CaCl_2$ solution. Before sorption studies, the beads were removed and allowed to dry at room temperature. The dried solid mass was stored in a polythene bag for further use (Mishra, 2013).

Biosorption experiments

The experiments were carried out in the batch mode for the measurement of adsorption capacities. From 100 ppm of cadmium metal ion solution, 50 ml was taken into a 250 ml conical flask and 0.2 g of the LLR was added corked with a rubber bung and shaken with a flask shaker for 2 h at room temperature (30°C) at 180 rpm. The separation of the adsorbents and solutions were carried out by filtration with whatman filter paper No 42 and the filtrates were stored in sample cans for use. The residual metal ions concentrations were determined using atomic absorption spectrophotometer (AAS). Pyeunicam Model SP. For studies on effect of temperature the adsorption studies were carried out at 25, 30, 40, 50, 60 and 70°C. The percentage adsorption was calculated using the following equation:

$$\%\text{Adsorption} = [(C_i - C_f/C_i)] \times 100 \quad (1)$$

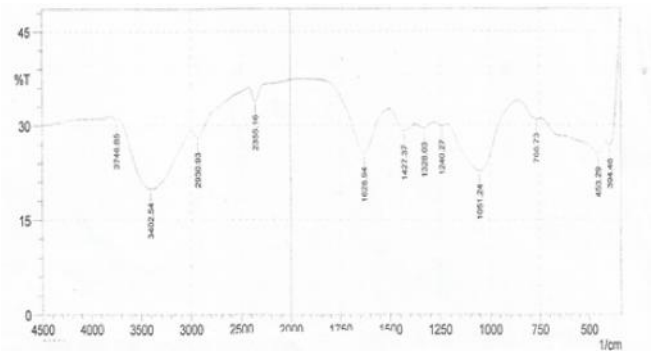


Figure 1. FTIR spectrum of *L. laxiflorus* root.

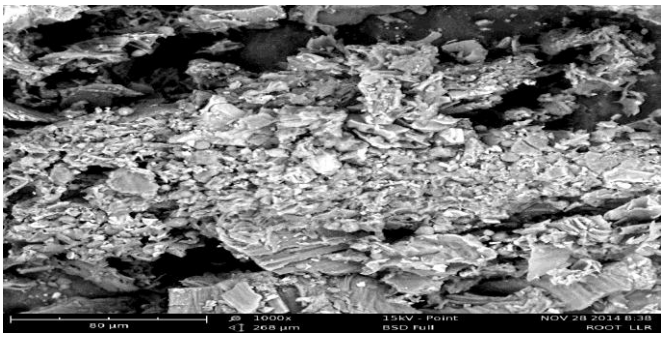


Figure 2. SEM of LLR powder in raw form.

Where C_i= Initial metal ion concentration and C_f = Equilibrium metal ion concentration (mg/L).

The amount of metal absorbed by the biosorbent was also calculated as

$$q_e = V(C_o - C_e) / M \quad (2)$$

Where, q is the amount of metal ion adsorbed in mg/g; C_o is the initial metal ion concentration in mg/cm^3; C_e is the final concentration in mg/cm^3, V is the volume of metal ion solution in liters; M is the mass of the root of *L. laxiflorus* powder used in gram.

RESULTS AND DISCUSSION

The FTIR is measured in the range of 400 to 4000 cm $^{-1}$ wave number. The FTIR of the adsorbent displays a number of adsorption peaks, indicating the complex nature of the studied adsorbent. The IR bands consisted of four regions, the broad OH band (3200-3600 cm^{-1}), C-H stretching region (2800-3000 cm^{-1}), carbonyl group stretching region (1550 -1750 cm^{-1}), and finger print bands (below 1550 cm^{-1}) (Shin et al., 2007).

From Figure 1, the absorption of peak at approximately 3400 were due to stretching vibrations of hydroxyl groups which are one of the main components of cell wall polysaccharides of the plants (Suantak et al., 2011). The adsorption peak at 2930 cm^{-1} is likely due to the presence of C-H asymmetric stretching vibration in –COOH group (Kumar et al., 2012) of methylene groups on the surface. The absorption band at 1630 cm^{-1} may be assigned to Amide I and II (protein) respectively (Pradhan et al., 2007). While the peaks in the range of 1427 to 1328 cm^{-1} could be attributed to carboxylate group (Pradhan et al., 2007). The broad peak at 1240 could be due to (C- C) or (C- H) or (C-O) stretching of the carboxyl groups (Singh et al., 2010). The peak in the region 1051 cm^{-1} is due to the presence of C- C Stretching of the polysaccharides (Singh et al., 2010). In conclusion, the FTIR spectroscopic analysis of the plant biomasses indicated the presence of hydroxyl, amide and carboxylate groups as the main functional groups involved in the complexation of metal ions for biosorption processes.

The adsorption capacity of the adsorbent depends upon porosity as well as chemical reactivity of the functional groups at the adsorbent surfaces. It seems that these functional groups participate in metal binding process.

Scanning electron microscope (SEM) analysis is another important tool used in the determination of the surface morphology of an adsorbent. The SEM image and micro- analysis of LLR plant is shown in Figure 2. The external surface of LLR is full of cavities with well developed porous structure. The external surfaces show a rough area having different pore diameters distributed over the surface of the biomass which may be responsible for metal removal. This analysis reveals a highly porous structure for LLR biomass. It could also be seen that more uneven and rough surface morphology exist in all the adsorbent.

X-ray diffraction (XRD) analysis was carried out by using XRD system with Cu-Kα radiation. The XRD patterns for LLR in raw powdered form is shown in Figure 3. These gives information about the changes in the crystalline and amorphous nature of the adsorbents. Sharp intensity XRD peaks have been observed at typical scanning angles of 2θ = 20. The sharp peaks present in the figures indicated the crystalline nature of the material. In addition, the presence of other weak intensity peaks in the spectra indicates the amorphous nature of the three adsorbents. The amorphous nature of the adsorbents suggests that metal ions can easily penetrate the surface which is desirable for an effective removal. These results are in good agreement with those reported Kugbe et al. (2009).

Effect of pH on metal biosorption

Hydrogen ion concentration is one of the important factors that influence the adsorption behavior of metal ions in aqueous solutions. It affects the solubility of metal ions in solution, replaces some of the positive ions found in active sites and affects the degree of ionization of the adsorbate during the process of biosorption. This is because it affects solution chemistry and also the

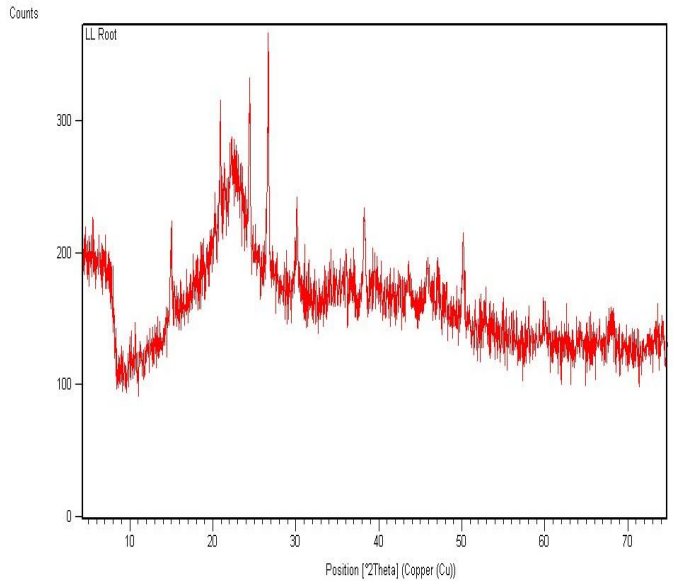


Figure 3. XRD image of *L. laxiflorus* roots raw powdered.

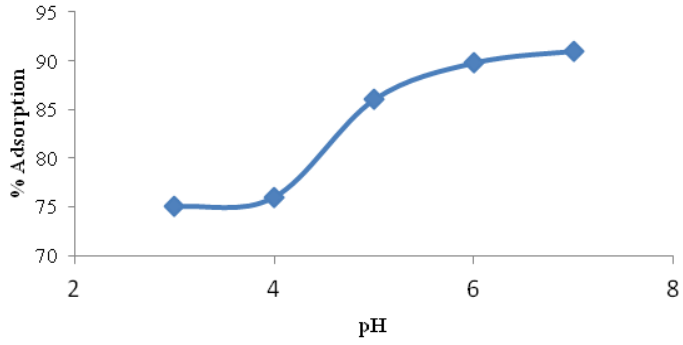


Figure 4. Effect of pH on % adsorption of Cd (II) ions on LLR biomass.

speciation of the metal ions. The effect of initial pH on biosorption of Cd (II) ions onto *L. laxiflorus* was evaluated in the pH range of 3.0 to 7.0. Studies in pH range above 7.0 were not attempted as there is precipitation of cadmium (II) hydroxides.

From Figure 4 it could be seen that Cd (II) ions adsorption increased as the pH increased. At low pH values, protons occupy the biosorption sites on the biosorbent surface and therefore less Cd (II) ions can be adsorbed because of electrostatic repulsion between the metal cations and the protons occupying the binding sites. When the pH was increased, the biosorbent surface became more negatively charged and the biosorption of the metal cations increased drastically until equilibrium was reached at pH 6.0 to 7.0. At pH of >7.0 there is formation of hydroxylated complexes of the metal ions and these complexes compete with the metal

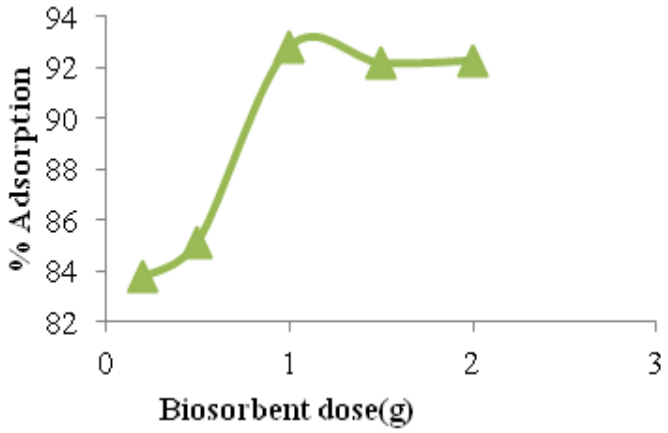


Figure 5. Effect of dosage on % adsorption of Cd (II) ions on LLR biomass.

cations for the adsorption sites hence a reduction in the effective metal cations removal. Therefore adsorption experiments at pH above this were not considered.

Effect of biosorbent dosage

The effect of biomass dosage on adsorption of Cd (II) ions is indicated in Figure 5. The number of available binding sites and exchanging ions for the biosorption depends upon the amount of biosorbent in the biosorption system. This is attributed to the fact that it determines the number of binding sites available to remove the metal ions at a given concentration. The dosage also determines the adsorption capacity of the biosorbent with an increase in mass reducing the biosorption capacity as the mass increase from 0.1 to 2.5 g per 20 ml of adsorbate. An increase in the % adsorption is attributable to an increase in the number of binding sites for the metal cations. Similar results were recorded in the literature for other adsorbents. However, the mass could not be increased infinitely as at some point all the solution is sequestered leaving no residual solution for concentration determination. Similar trend have been found by Mahajan and Sud (2011).

Effect of initial metal concentration

The initial concentration remarkably affected the uptake of Cd (II) ions in solution. The efficiency of Cd (II) ions adsorption by LLR at different initial concentrations (10 to 80 mg L^{-1}) was investigated as shown in Figure 6. At a lower concentration, the adsorption sites take up the available metal ions much quickly due to less competition among the metal ions for the available binding sites which are fixed in this case. However, as the concentration increases the competition for the limited

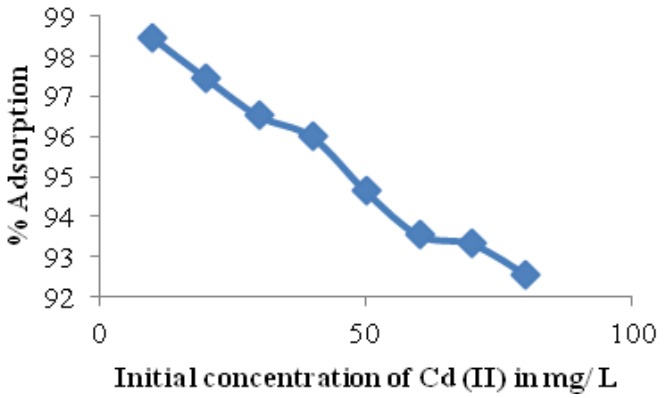


Figure 6. Effect of initial cadmium concentration on adsorption of LLR biomass.

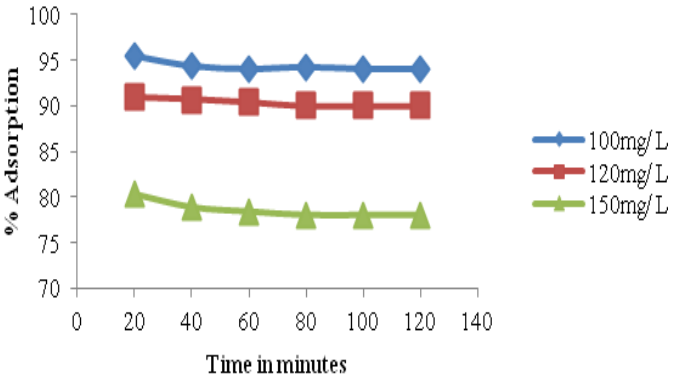


Figure 7. Effect of contact time on adsorption of Cd (II) ions by LLR.

binding sites sets in as the binding sites become saturated (Mahajan et al., 2013).

Effect of contact time

Contact time is an important parameter for any successful use of the biosorbents for practical purposes. Effect of contact time on adsorption of Cd (II) ions was investigated keeping the biomass in contact with the metal ion solution for different time periods between 0 to 60 min. It was noted that as adsorption proceeds, the sorbent reaches saturation state, at this point the sobbed solute tends to desorbs back into solution (Figure 7). Eventually, the rate of adsorption and desorption are equal at equilibrium. When the system attains equilibrium, no further net adsorption occurs. The time taken to attain equilibrium is very important for process optimization. The rate of adsorption is very fast at first and over 95% of total biosorption of Cd (II) ions occurs in the first 5 min and thereafter it proceeds at a slower rate

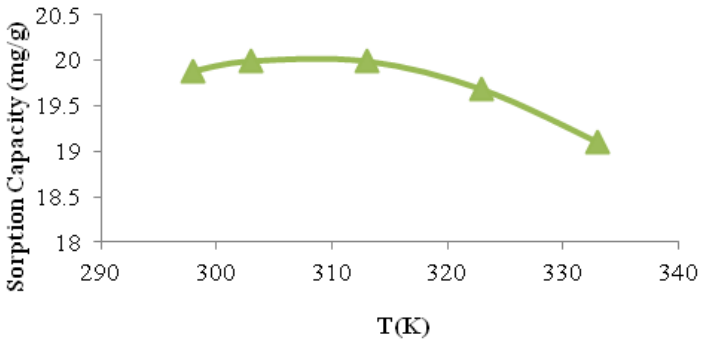


Figure 8. Effect of temperature on % adsorption of Cd (II) ions by LLR.

and finally no further significant adsorption is noted beyond 20 min of contact time. The very fast adsorption makes the material suitable for continuous flow water treatment systems (Sarin and Paint, 2006).

Effect of temperature

Temperature of the medium affects the removal efficiency of pollutants in aqueous solutions. This is because a change in temperature in turn affects the solubility of pollutants and also the kinetic energy of the adsorbing ions. Therefore the effect of temperature on adsorption of Cd (II) ions was investigated and the data is shown in Figure 8. The results indicate that the percentage adsorption increases with increase in temperature up to 40°C, after that any increase in temperature is accompanied by a reduction in % adsorption. This can be attributed to the fact that with increase in temperature of the solution, the attractive forces between the biomass surface and Cd (II) ions are weakened thus decreasing the sorption efficiency. This could be due to increase in the tendency for the Cd (II) ions to escape from the solid phase of the biosorbent to the liquid phase with increase in temperature. Finally increased temperature beyond 40°C could have destroyed some of the binding sites on the biosorbent surface due to bond rupture (Meena et al., 2005).

Biosorption kinetics

Kinetic study provides useful information about the mechanism of adsorption and subsequently investigation of the controlling mechanism of biosorption as either mass transfer or chemisorption. This helps in obtaining the optimum operating conditions for industrial-scale batch processes. A good correlation of the kinetic data explains the biosorption mechanism of the metal ion on the solid phase (Garima and Dhiraj, 2013). In order to

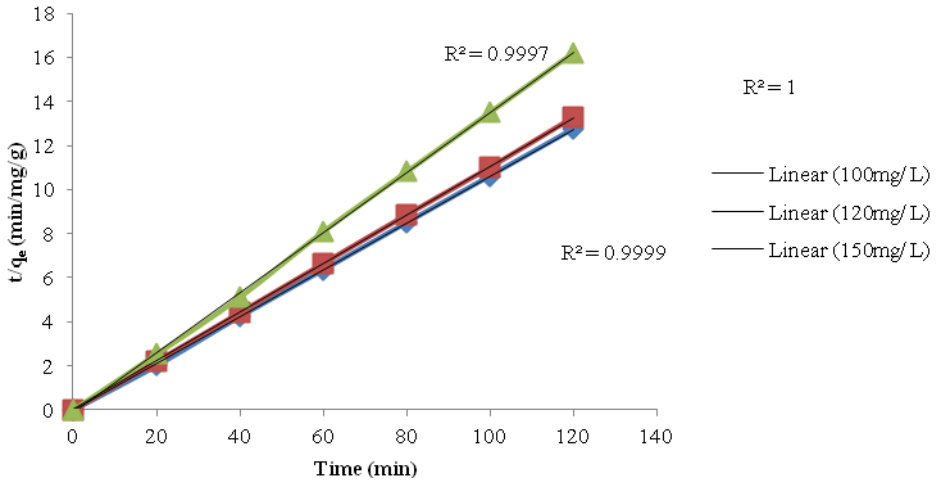


Figure 9. Pseudo-second -order- plots of Cd (II) adsorption onto LLR.

evaluate the kinetic mechanism that controls the biosorption process, the pseudo -first-order models (Lagergren, 1898) were applied for biosorption of Cd (II) ions on the biosorbent. The Lagergren pseudo- first – order rate model is represented by the equation:

$$\log qe - qt = \log qe - \frac{K1}{2.303}t \quad (3)$$

Where q_e and q_t are the amounts of metal adsorbed (mg g^{-1}) at equilibrium and at time t respect ively, and K_1 is the rate constant of pseudo-first-order biosorption (min $^{-1}$). The q_e and rate constant were calculated from the slope and intercept of plot of log (q_e- q_t) against time t.

The pseudo-second-order equation (Ho and Mckay, 1999) assumes that the rate limiting step might be due to chemical adsorption. According to this model metal cations can bind to two binding sites on the adsorbent surface. The equation can be expressed as shown below:

$$\frac{t}{q_t} = \frac{1}{K_2 q_e^2} + \frac{1}{q_e^t} \quad (4)$$

Where k_2 is the rate constant of the pseudo-second-order adsorption (g/mg/min). If the adsorption kinetics obeys the pseudo-second-order model, a linear plot of t/q t versus t can be observed as shown in Figure 9.

Biosorption isotherms

For optimization of the biosorption process design, it is imperative to obtain the appropriate correlation for the equilibrium data. Biosorption isotherms describe how adsorbate interacts with the biosorbent and the residual metal ions in solution during the surface biosorption. The isotherms also help in determination of adsorption capacity of the biosorbent for the metal ions. The data on Cd (II) ions biosorption was fitted with the Langmuir (1918) and Freundlich (1906) isotherms (Figures 10 and 11).

The Langmuir isotherm assumes monolayer coverage of the adsorbate onto a homogeneous adsorbent surface and the biosorption of each cation onto the surface has equal activation energy. The Langmuir isotherm can be exppressed as:

$$\frac{C_e}{q_e} = \frac{1}{q_{max} b+} + \frac{C_e}{q_{max}} \quad (5)$$

Where qmax is the monolayer capacity of the biosorbent (mg g^{-1}), and b is the biosorption constant (L mg $^{-1}$). The plot of ce/qe versus ce should be a straight line with a slope of 1/qmax and intercept of 1/q max b when the biosorption follows Langmuir equation. The Freundlich equation can be expressed as:

$$\log q_e = \log K_f + \frac{1}{n} \log C_e \quad (6)$$

Where K_f and 1/n are the Freundlich isotherm constants related to biosorption capacity and biosorption intensity respectively. If the equation applies then a plot of log qe versus log Ce will give a straight line of slope 1/n and intercept as K_f

LLR has a higher value for n suggesting multilayer sorption on the surface of the plant biomass, which is due to its various chemical functional groups. The values of b and K obtained for LLR also confirms a higher sorption capacity and superior performance of LLR adsorbents for Cd (II) ions adsorption from aqueous solution. Similar results are reported by Mahajan et al. (2013), Iqwe et al. (2005) and Kurniawan and Thiam (2010).

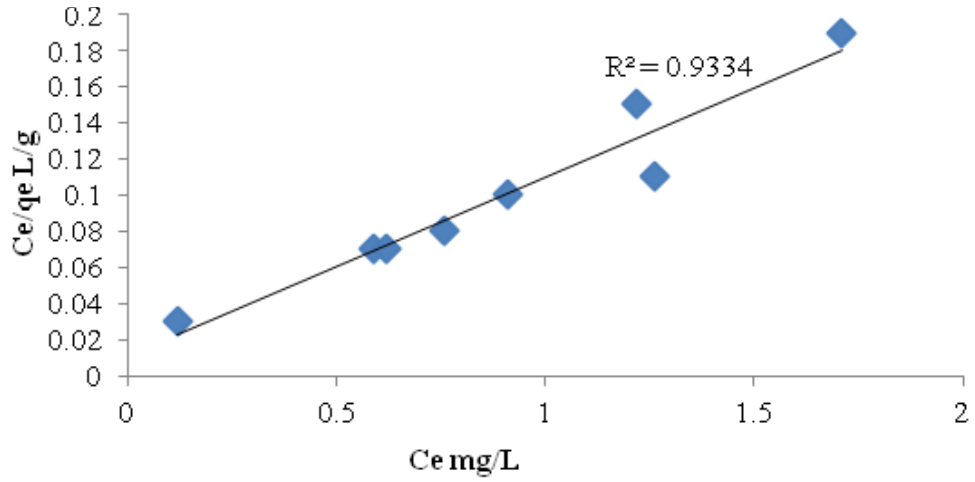


Figure 10. Langmur isotherm for the sorption of cd(II) using LLR.

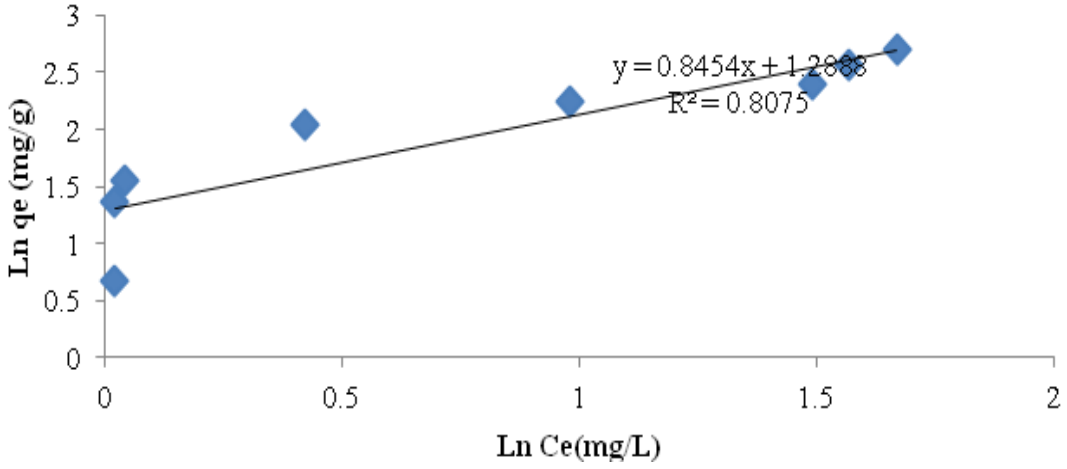


Figure 11. Freundlich adsorption isotherm for cadmium by LLR.

Conclusion

Adsorption of cadmium ions from aqueous solution using the *Lonchocarpus laxiflorus* plant roots material was investigated. Various contributing parameters such as contact time, initial metal ion concentration, solution pH, and adsorbent dose was optimized for maximum removal efficiency. The sorption data fitted well with Langmuir isotherm with high R^2 values. The kinetic studies indicated that the pseudo second order model was the best one in describing the kinetics of cadmium (II) adsorbed onto roots powder. A large number of carbonyl and hydroxyl groups were observed in the FTIR analysis, XRD studies reveal the crystalline structure of the biosorbent and SEM studies showed the presence of various moieties that enhances the adsorption phenomenon. Excellent removal efficiency in its encapsulated form explores the utilization of the biomass at the commercial scale for small scale industries, making it of potential commercial use.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

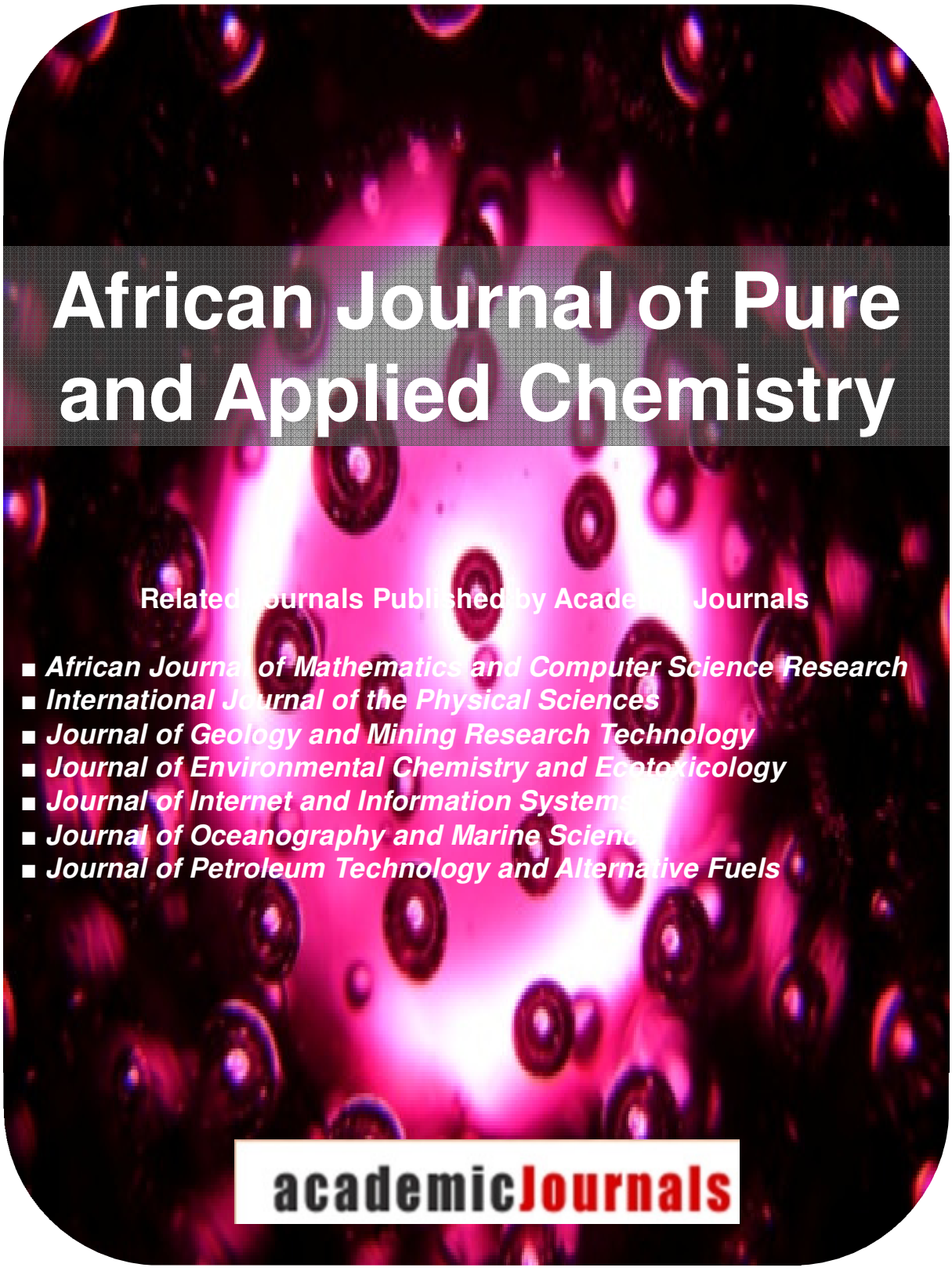
Ajay KM, Mishra KJ, Rai PK, Chitra R, Nagar PN (2005). Removal of heavy metal ions from aqueous solution using carbon aerogel as an adsorbent. Indian J. Chem. Technol.10:72-78.

Babel S, Kurniawan TA (2003). Low-cost adsorbents for heavy metals uptake from contaminated water: A review. J. Hazard. Mater. 97(1-3):219–243.

Bailey SE, Olin TJ, Bricka RM, Adrian DD (1999). A review of potentially low-cost biosorbents for heavy metals. Water Res. 33:2469-2479.

Davis TA, Volesky B, Mucci A (2003). A review of the biochemistry of

heavy metals biosorption by brown algae. Water Res. 37(18):4311-4330.
Feng N, Guo X, Liang S, Zhu Y, Liu J (2010). Biosorption of heavy metals from aqueous solutions by chemically modified orange peel. J. Hazardous Mater.185:49-54.
Fouad K, Noureddin A, Mohammed CN (2012). Adsorptive removal of cadmium from aqueous solution by cork biomass. Equilibrium, dynamic and thermodynamic studies. Arab. J. Chem. Doi:10.1016/J. arabjc.2011.12.013
Freundlich HMF (1906). Uber die adsorption in lasugen. J. Phys. Chem. 57:385–470.
Garima M, Dhiraj S (2013). Accessing the Potential of Lingocellulosic Agricultural Waste Biomass for Removal of Ni (II) Metal Ions from Aqueous Streams. Int. J. Sci. Eng. Res. 4(40):1713-1720.
Gupta VK, Nayak A (2012). Cadmium removal and recovery from aqueous solutions by novel adsorbents prepared from orange peel and Fe_2O_3 nanoparticles. Chem. Eng. J. 180:81–90.
Ho YS, McKay G (1999). Pseudo—second order model for sorption processes. Process Biochem. 34:451–465.
Igwe JC, Abia AA (2006). A bioseparation process for removing heavy metals from wastewater using biosorbent. Afr. J. Biotechnol. 5(12):1167-1177.
Kugbe J, Matsue N, Henmi T (2009). Synthesis of Linde Type A Zeolite-Goethite Nanocomposite as an Adsorbent for Cationic and Anionic Pollutants. J. Hazardous Mater. 164(2-3):929-935.
Kumar KK, Prasad MK, Sarada B, Murthy CVR (2012). Studies on biosorption of Nickel using immobilized fungus. *Rhizomucor tauricus*. Bioresour. 7(4):5059-5073.
Kurniawan K, Thami Larabu P (2010). Removal of iron (111) from aqueous solution using Ricinus communes Seed Shell and polypyrole coated Ricinus communes seed shell activated carbon. Int. J. Chem. Tech. Res. CODEN (USA): IJCRGG. 2(1):26-35.
Lagergren S (1898). Zur theorie der sogenannten adsorption gelöster stoffe, K. Sven. Vetenskapsakad. Handl. 24:1-39.
Langmuir I (1918). The adsorption of gases on plane surfaces of glass, mica and platinum. J. Am. Chem. Soc. 40:1361–1403.
Mahajan G, Umesh G, Vinod G (2013). Utilization Properties of Jatropha De- oiled cake for removal of Nickel (ii) from aqueous solutions. BioResources 8(4):5596-5611.
Mahajan G, Sud D (2011). Kinetics and equilibrium studies of chromium (vi) metal ion remediation by Arachis hypogeal shells. Agreen approach. BioResources 6:3324-3338.
Meena AK, Mishra GK, Rai PK, Rajagopal C, Nagal PN (2005). Removal of heavy metal ions from aqueous solutions using carbon aerogel as an adsorbent. J. Harzadous Mater. 122:161-170.
Mishra SP (2013). Adsorption of Cu and Zn on calcium alginate immobilized penicillium sp. Indian J. Chem. Technol. 20:21-25.
Nida MS, Ahlam MF, Aklam A (2012). Biosorption of Cadmium(11) from Aqueous Solution by *Prunus avium* Leaves. Am. J. Environ. Eng. 2(5):123-127.
Orhan Y, Buyukgungor H (1993). The removal of heavy metals by using agricultural wastes. Water Sci. nigricans. Water Res. 32:1437-1444.
Osemeahon SA, Esenowo DI (2012). Evaluation on the application of immobilized Konkoli (*Maesopsis eminii*) immobilized leaves for the removal of Pb^{2+} Cd^{2+} and Zn^{2+} in aqueous solution. Afr. J. Pure Appl. Chem. 6(5):59–64.
Pradhan J, Das SN, Thaker RS (2007). Adsorption of hexavalent chromium from aqueous solution by using activated red mud. J. Colloid Interf. Sci. 217(1):137–141.
Santhi IT, Manonmani S (2012). Adsorption of Methylene Blue from aqueous Solution onto a waste aquacultural Shell Powders (prawn waste). Sustain. Environ. Res. 22(1):45-51.
Sarin V, Paint KK (2006). Removal of chromium from indusrial waste water by using eucalphus bark. Bioresource Technol. 97: 15–20.
Shin EW, Karthikeyan KG, Tshabalala MA (2007). Adsorption mechanism of cadmium on juniper bark and wood. Bioresour. Technol. 98:588-594.
Singh R, Chadetric R, Kumar R, Bhatia D, Kumar Bishnoi NR, Singh N (2010). Biosorption Optimization of lead II, Cadmiun II and Copper II using response surface methodology and applicability in isotherm and thermodynamics modeling. J. Hazard. Mater. 174:623-634.
Suantak KC, Baloma J, Shri C (2011). Removal of arsenic (iii) from aqueous solution by biosorption on to maize (*Zea mays*) leaves surface: parameters optimization sorption isotherms, kinetics and thermodynamic studies. Res. J. Chem. Sci. 1(5):73–79.
World Health Organisation (Ed). (2008). Guidelines for drinking water quality, world health organization, ISBN 9241544743, Geneva.


African Journal of Pure and Applied Chemistry
Related Journals Published by Academic Journals
African Journal of Mathematics and Computer Science Research
International Journal of the Physical Sciences
Journal of Geology and Mining Research Technology
Journal of Environmental Chemistry and Ecotoxicology
Journal of Internet and Information Systems
Journal of Oceanography and Marine Science
Journal of Petroleum Technology and Alternative Fuels
academicJournals