Archives of Current Research International
7(1): 1-7, 2017; Article no.ACRI.31929
ISSN: 2454-7077

SCIENCEDOMAIN *international*
www.sciencedomain.org

Evaluation of the Antimicrobial Activity and Chemical Composition of the Leaf Extract of *Annona muricata Linn* (Soursop) Grown in Eastern Nigeria

Rosemary I. Uchegbu[1*], Kalu U. Ukpai[2], Irenus C. Iwu[3] and Jacinta N. Akalazu[4]

[1]Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.
[2]Department of Biology, Alvan Ikoku Federal College of Education, Owerri, Imo State, Nigeria.
[3]Department of Chemistry, Federal University of Technology, Owerri, Imo State, Nigeria.
[4]Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author RIU designed the study, determined the phytochemical and mineral constituents of the sample, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author KUU performed the anti –bacterial analysis. Author ICI determined the mineral and vitamin analysis of the study. Author JNA performed the antifungal analysis of the study and managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ACRI/2017/31929
Editor(s):
(1) Magdalena Valsikova, Horticulture and Landscape Engineering, Slovak University of Agriculrure, Nitra, Slovakia.
Reviewers:
(1) Jeferson Júnior da Silva, Piracicaba Dental School, University of Campinas – UNICAMP, Brazil.
(2) Lorna T. Enerva, Polytechnic University of the Philippines, Philippines.
(3) Monthon Lertcanawanichakul, Walailak University, Nakhon-Si-Thammarat 80160, Thailand.
(4) Eliton da Silva Vasconcelos, Federal University of São Carlos, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/18212

Original Research Article

Received 30th January 2017
Accepted 6th March 2017
Published 15th March 2017

ABSTRACT

Aim: This work was carried out to test the inhibitory ability of the plant extract on some human pathogens, to identify the fungi associated with yam tuber rot in Nigeria, to evaluate the antifungal activity of methanol extract of *Annona muricata* (30 mg/ml) leaf along with mancozeb (0.3 g/ml on mycelial growth inhibition of fungi isolated from *Dioscorea rotundata* (poir) (white yam) and to determine the phytochemicals and nutritive values of *Annona muricata* leaf.
Study Design: The study was designed to test the inhibitory ability of the extract on human and plant pathogens, to determine the phytochemicals and nutritive values of *Annona muricata* leaf.

**Corresponding author: Email: mrsriu@yahoo.com;*

Place and Duration of Study: Department of Chemistry, Alvan Ikoku Federal College of Education, Owerri and Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria during February to July 2016.

Methodology: Standard assay techniques were used to evaluate the leaves for nutrients and phytochemical composition. The antibacterial activity was performed by filter paper disc diffusion technique. Antifungal susceptibility test against the fungal isolates were performed by disc diffusion method.

Results: The ethanol extract inhibited all the tested organisms *E. coli, K. pneumoniae*, *S. aureus*, *P. mirabilis* and *Salmonella*. The antifungal results showed that *Aspergillus flavus, Fusarium oxysporium, Rhizopus stolonifer* and *Botryodiploidia theobromae* were associated with yam tuber rot. The results revealed that *Annona muricata* inhibited the growth of the test organisms with different inhibition zones. The most devastating fungus was *B. theobromae*, causing rot of 9.50 cm^2, this was followed by *R. stolonifer*, causing rot of 7.50 cm^2 while the least devastating was *F. oxysporium* (7.31 cm^2). *In vivo* results showed that *Annona muricata* leaf extract was effective in reducing the yam tuber rot. The phytochemical studies of *Annona muricata* revealed the presence of alkaloids (1.66%), flavonoids (1.15%), tannins (0.75%), phenols (0.14%) and saponins (2.15%). The leaves contain some minerals such as calcium (Ca) (3.87 %), magnesium (Mg) (1.07%), potassium (K) (0.30%), sodium, (Na) (0.26%), phosphorus (P) (0.30%) and nitrogen,(N) (3.89%). The plant samples were found to be rich in vitamins comprising riboflavin (0.11 mg/100g), thiamin (0.15 mg/ 100 g), niacin (0.25 mg/ 100 g) and ascorbic acid (8.20 mg/ 100 g). The proximate composition revealed the presence of protein (24.3%), crude fibre (6.17%), fats/oil (2.52%), ash (5.30%), carbohydrates (61.7%) and food energy (366.7 g/cal). The leaves contain appreciable quantities of proteins, fiber, vitamins and minerals which have health promoting benefits. The results of the analysis justify the use of *Annona muricata* as a good food and a potent drug.

Keywords: Annona muricata; tuber rot; pathogens; deterrents; diseases.

1. INTRODUCTION

Annona muricata is a small erect evergreen tropical plant belonging to the family Annonaceae, growing 5-6 meters in height. The leaves, bark, fruits and roots of the *Annona muricata* trees are used as ingredients in various traditional herbal medicines. The fruit and the leaves are used in traditional medicine for their tranquillizing and sedative properties. Decoction of the leaves of *A. muricata* works as a pain reliever and helps cure gall bladder diseases traditionally. The leaves can be applied topically to get rid of eczema, skin rash and swelling. Topical application of these leaves promotes fast healing of wounds and prevents infections. The fruits are used to reduce joint pain, to treat heart conditions, as a sedative and to reduce coughing or flu symptoms in herbal medicines. Herbalists believe that *Annona muricata* can cure cancer (if detected in earlier stage). Its benefits for cancer involves killing of cancer cells without damaging the healthy cells, thus it can be a good alternative for chemotherapy [1]. Studies showed that *A. muricata* is beneficial in treating nearly twelve different types of cancer, including most common cancer like colon, breast, lung, pancreatic cancer etc. It was reported that the immune system of the cancer patients who consumed soursop during chemotherapy cycles were not affected or weakened much, as compared to other cancer patients [2]. Drinking boiled leaves of *A. muricata* increases appetite and inhibits abnormal growth of specific cells such as free radicals but does not damage healthy cells.

In Jamaica and West Indies, the leaves are used as tea to reduce catarrh, and in the treatment of liver diseases, urological problems such as bed wetting or frequency and to help sleep [2]. The black seeds are crushed and used as vermifuge. All parts of the tree might be ground and used as a sedective or as an anti-convulsant [3]. The wound healing activity of alcoholic extract of stem and bark of *Annona muricata* was found to show the marked reduction in area of the wound which was tested in the albino rats which proves their possible use in the healing wound [1,4]. Antibacterial analysis carried by [4] revealed that the methanolic and aqueous extract of the leaves of *Annona muricata* tested against various bacterial strains such as *Staphylococcus aureus* ATCC29213, *Escherichia coli* ATCC8739, *Proteus vulgaris* ATCC13315, *Streptococcus pyogenes* ATCC 8668, *Bacillus subtilis* ATCC12432, *Salmonella typhimurium* ATCC 23564, NCIM No.2719 *Enterobacter aerogenes*

NCIM No. 2340 and *Klebsiella pneumonia* No.2719 showed positive activities against tested organisms.

Fungi are reported to produce mycotoxins which contaminate food and feedstuff such as yam. Synthetic chemicals used in plant disease control have been implicated with environmental pollution and toxicity to human. Phytochemicals are reported to be ecofriendly, non poisonous to man and therefore a better alternative to plant disease control [5]. Hence this work is carried out to test the antifungal potency of the methanol extract of the leaves of *Annona muricata,* its nutrient and anti- nutrient compositions.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fresh leaves of *A. muricata* were collected from Umudike in Umuahia, Abia State, Nigeria. The leaves were allowed to dry in a laboratory bench for 10 days. The dried leaves were milled into powder (400g) using Thomas Willey Machine (Model 5 USA), stored in a tight bottle and used for analysis.

2.2 Chemical Analysis

The phytochemicals were determined according to the method described by [6]. The macro elements, calcium, sodium, potassium, phosphorus, magnesium were determined according to the method described by [7].The vitamin C and B complexes (thiamin, riboflavin and niacin) were determined according to [7]. Total nitrogen (N) content was determined by the use of a micro Kjeldahl MD 55 (Singapore) apparatus [7]. The protein content was calculated as N x 6.25. Crude fiber, Fats/oil and ash content were determined according to [8]. Total Carbohydrates were estimated as the remainder after accounting for ash, crude fiber, Protein and fats/oil and the gross food energy was estimated according to the method of [8] by using the equation.

$$FE = (\% CP \times 4) + (\% CHO \times 4) + (\% Fat \times 9)$$

Where

FE = Food energy (in g / cal)
CP = Crude Protein
CHO = Carbohydrates

2.3 Antibacterial Evaluation of the Leaves of *A. muricata*

2.3.1 Preparation of extracts

The test solution of each extract was prepared by dissolving 0.1g of the plant extracts separately in 1.0 ml of dimethylsulphoxide (DMSO) to get a concentration of 100 mg/ml.

2.3.2 Micro-organisms

The bacteria organisms used were *Staphylococus aureus*, *Klebsiella pneumonia*, *Salmonella*, *Proteus mirabilis and Escherichia coli.* All the organisms were obtained from the stock culture of the Federal Medical Center, Umuahia. Cultures were brought to laboratory conditions by resuscitating the organisms in peptone water and thereafter subcultured into nutrient agar medium and incubated at 37°C for 24 hours.

2.3.3 Antibacterial assay

The antibacterial activity was performed by filter paper disc diffusion technique. Filter paper disc (whatman No1, 6mm diameter) were placed in glass petri dishes and sterilized in hot air oven [9]. The media (10 g nutrient agar in 200 ml distilled water, auto-claved at 115°C for 30 minutes) was cooled to 50°C. The sterile nutrient agar media were poured into the sterile petri dishes and allowed to solidify. The bacteria were swabbed with a sterile wire loop [10]. Each disc was impregnated with 0.2 ml of plant extracts and standard- Ciprofloxacin. Discs with DMSO (100 mg/ml) served as a control.

The discs were used after drying them in an incubator at 40°C to remove any trace of solvent [10]. Discs were introduced onto the surface of the medium. The plates were incubated at 37°C for 24 hours to obtain zones of inhibition. The experiments were repeated three times for each extract and twice for reference antibiotic to minimize error and the average of these values were tabulated.

2.3.4 Antifungal assay

Three pieces of infected, dried and sterilized yam tuber discs (2 mm) were plated on potato dextrose agar (PDA) and incubated at 27°C. Dried leaf extract of *Annona muricata* was prepared by grinding to powder. Following the procedures of [11], about 4 kg of powdered

sample was soaked in 98% ethanol for 4days. Then 20 g of the ethanolic extract was soaked in methanol for 48hours and filtered through what man filter paper. The filtrate was concentrated with rotary evaporator at 40°C to a dark brown extract.

Sterile filter paper discs (6mm) impregnated with the *Annona muricata* leaf extract was carefully and firmly placed on PDA plates earlier seeded with each fungal pathogen.

For *in vivo* study, yam discs (1 cm thick) were removed from the tubers, then 1 ml of methanol extract was dispersed into each hole. In control 1ml sterile water was dispersed into the holes. The holes were replaced with yam discs initially removed and then sealed with Vaseline.

2.4 Statistical Analysis

All values are expressed as mean ± S.D. Statistical analysis were performed by Student's *t*-test. The values of p lower than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

Phytochemical analysis of the leaves of *A. muricata* revealed the presence of alkaloids, flavonoids, tannin, saponins and phenols. Pure isolated plant alkaloids and their synthetic derivatives are known for their analgesic, antipasmodic and antibacteriacidal effects. Alkaloids are also reported to have anti-tumourous effect [11]. *A. muricata leaves* contain alkaloid (1.66 %), this may be attributed to the reason decoction of the leaves of *A. muricata* works as a pain reliever.

Table 1. Phytochemical composition of leaves of *Annona muricata* expressed as percentages (%)

Constituents	Leaves
Alkaloids	1.66 ± 0.35
Flavonoids	1.15 ± 0.20
Tannins	0.75 ± 0.02
Phenols	0.14 ± 0.01
Saponins	2.15 ± 0.05

Values are means of triplicate determinations ± standard error

A. muricata leaves were found to contain 1.15% of flavonoid. Flavonoids are known to prevent oxidative cell damage, have strong anti-cancer activity and inhibit all stages of carcinogenesis. Flavonoids possess antioxidant property, protect against allergies, inflammation, microbes, ulcer, viruses and tumor [12]. The leaves of *A. muricata* can be applied topically to get rid of eczema, skin rash and swelling traditionally. This also supported the use of *A. muricata* by the natives for the treatment of cancer.

Table 2. Mineral composition of leaves of *Annona muricata* expressed in percentages (%)

Elements	Leaves
Magnesium	1.07 ± 0.10
Calcium	3.87 ± 0.23
Potassium	0.30 ± 0.05
Sodium	0.26 ±0.30
Phosphorus	0.30 ± 0.20
Nitrogen	3.89 ± 0.15

Values are means of triplicate determinations ± standard error

Table 3. Vitamin composition of leaves of *Annona muricata* (mg/100 g)

Constituents	Leaves
Riboflavin	0.11 ± 0.35
Thiamin	0.15 ± 0.05
Niacin	0.25 ± 0.50
Ascorbic acid	8.20 ± 0.20

Values are means of triplicate determinations ± standard error

Table 4. Proximate composition and energy content of leaves of *Annona muricata*

Constituents	Leaves
Crude Protein N x 6.25 %	24.3 ± 0.10
Crude Fiber %	6.17 ± 0.30
Fats/oil %	2.52 ± 0.15
Ash %	5.30 ± 0.50
Carbohydrates %	61.7 ± 0.17
Food energy g/cal	366.7 ± 0 40

Values are means of triplicate determinations ± standard error

The leaves of *A. muricata* contain tannin (0.75%). Tannins are organic substances of diverse composition with pronounced astringent properties that promote the healing of wounds and inflamed mucous membranes [13,12]. Externally, the leaves of *A. muricata,* promote fast healing of wounds and prevent infections. A correlation has been made between oesophageal or nasal cancer in humans and regular consumption of certain herbs with high tannin concentrations.

A. muricata leaves contain phenols (0.14%). Plants that contain phenols could be used as anti-inflammatory, immune enhancers and hormone modulators [13].

Saponins are known to make the bronchial secretion more liquid, reduce the congestion of the bronchi and ease coughing. The leaves of *A. muricata* contain saponins (2.15%). Report showed that the fruits of *Annona muricata* are used to to treat heart conditions, as a sedative and to reduce coughing or flu symptoms in herbal medicines. From this analysis it shows that the leaves can also be used to treat bronchi problems and cough.

The leaves of *Annona muricata* contain important minerals such as Calcuim (3.87%), Magnesium (1.07%), Potassium (0.30%), Sodium (0.26%), Phosphorus (0.30%) and Nitrogen (3.89%). Phosphorus is required for nearly every metabolic process in the body .It is good for kidney function. Magnesium helps to reduce cholesterol according to [14]. It is good for human health as it is known to reduce blood pressure. Na and K are needed in the blood fluid and in nerves.

Calcium is a major factor for sustaining strong bones and plays a part in muscle contraction and relaxation, blood clotting, synaptic transmission and absorption of vitamin B12, large amounts of calcium are needed to make bone. Thus, substantial amounts are needed in the diet, especially during infancy, childhood, and pregnancy [15,16].

A. muricata leaves also contain essential vitamins. Vitamin C is an anti-scurvy vitamin. It facilitates the transformation of cholesterol into bile acid in the liver. The presence of vitamin C hastens the healing of wounds. It enhances the absorption of iron and thus has a role in reducing iron deficiency and anemia. It is necessary for healthy teeth, gums and bones and is essential for proper functioning of adrenal and thyroid glands.

Other vitamins such as niacin, riboflavin and thiamin were also found in the plant. Niacin is active in preventing the diseases pellagra which is characterized by skin and mucous membrane disorders as well as depression and confusion.

The results of the proximate composition showed that *A. muricata* leaves contain basic food nutrients such as protein, fats, carbohydrates and fiber. Fiber is very important for vibrant health. The important role of fiber is to clean out or sweep the digestive system, flushing the residue as efficiently and quickly as possible [12].

Table 5. Inhibition Zone Diameter (IZD) (mm) of *Annona muricata* leaves on the human pathogens

Pathogens	IZD of	*A. muricata* leaves
Proteus mirabilis	9.0 ±	0.20
Klebsiella pneumoniae	13.0 ±	0.45
Staphylococus aureus	10.0 ±	0.10
Salmonella	10.0 ±	0.10
Escherichia coli	11.0 ±	0.30

Values are mean of triplicate determination ± standard error

Table 6. *In vitro* inhibitions of fungal pathogens by *A. muricata* leaf extract (mm)

Pathogens	Methanol 30 mg/l	Mancozeb 030 g/ml
Aspergillus flavus	7.37± 0.50	21.00 ± 0.32
Fusarium oxysporium	9.10 ± 0.52	19.00 ± 0.32
Botryodiplodia theobromae	17.20 ± 0.52	22.60 ± 0.40
Rhizopus stolonifer	12.0 ± 0.06	23.00 ± 0.30

Table 7. *In vivo* yam rot reduction by *A. muricata* leaf extract

Dimensions of rot reduction (cm^2)				
Treatments	***A. flavus***	***F. oxysporium***	***B. theobromae***	***R. stolonifer***
Methanol	5.30 ± 0.20	4.40 ± 0.30	7.60 ± 0.30	6.20 ± 0.32
Mancozeb (0.3 g/ml)	0.00	0.00	0.40 ± 0.60	0.00
Water (control)	6.50 ± 0.36	5.00 ± 0.50	9.50 ± 0.40	7.50 ± 0.230

Ethanolic extracts of the leaves of *Annona muricata* exhibited antibacterial activities on the pathogens tested as shown in Table 5. The extracts inhibited all the tested organisms *S. aureus, P. mirabilis, K. pneumoniae*, salmonella and *E. coli*. This work is in agreement with the work of Pathak et al. [4] and Gajalakshmi et al. [3] who in their different studies reported that methanol and aqueous extract of *A. muricata* inhibited all the tested organisms. The organisms *P. mirabilis* and *E. coli* are the common cause of urinary tract infection and traveler's diarrhea [17]. Salmonella bacteria cause typhoid fever and intestinal infections which include diarrhea, abdominal pain, fever, nausea and vomiting. Diarrhea from salmonella poisoning can be severe enough to cause extreme dehydration requiring hospitalization along with inflammation of the intestinal wall [18]. Severe eye infections such as blepharo conjuctivities, corneal ulcers, abscesses, styes, dacryocystitis, orbital cellutitis and blebs are mainly caused by *Staphylococus aureus* and *Pseudomonas aeruginosa* [19,20].

The results of the antifungal study showed that, *A. flavus. R. stolonifer, Fusarium oxysporium* and *B. theobromae* were fungi associated with yam tuber rot.

Antifungal potency of *Annona muricata* against the isolated fungi showed that the leaf extract inhibited the growth of these fungi. *Annona muricata* was reported to inhibit the growth of *Bacillus subtilis, S.aureas, Streptococcus pyrogenes, Klebsiella pneumonae, Candida albicans* [21]. *Annona muricata* was reported to contain acetogenins which possess antimicrobial, anti tumour, antimalarial and insecticidal activities [3].

4. CONCLUSION

Result of the analysis showed *Annona muricata* leaves as good sources of both essential nutrients and phytochemicals. Most of the secondary metabolites identified in the test samples like flavonoids, saponins, tannin, phenols and alkaloids are phytoprotectants and are important for cell growth, replacement, and body building. The presence of these compounds in the extract of this plant indicates that it has radical-scavenging activity and may be the reason for popularly reported high health beneficial properties of the plant. The leaves contain various pharmacologically active compounds. The result of the antibacterial activities of this plant showed that the leaves of *Annona muricata* can be used for the treatment of the diseases caused by the bacteria. *Annona muricata* leaves can also be helpful in treatment of mycotic infections and in the control of fungal plant diseases.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Padmaa PM, Chansouria JPN, Khosa RL. Wound healing activity of *Annona muricata* extract. Journal of Pharmacy Research. 2009:2(3):404-406.
2. McLaughlin, Chih HW; 2000. Available:www.wisegeek.com
3. Gajalakshmi S, Vijayalakshmi S, Devi Rajeswari V. Phytochemical and pharmacological properties of *annona muricata*: A review. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(2):1-6.
4. Pathak P, Saraswathy D, Vora A, Savai J, *In vitro* antimicrobial activity and phytochemical analysis of the leaves of *Annona muricata*. International Journal of Pharma Research and Development. 2010;2(5).
5. Adewole SO, Ojewole JAO. Protective effects of *Annona muricata* Linn. (Annonaceae) leaf aqueous extract on serum lipid profiles and oxidative stress in hepatocytes of streptozotocin-treated diabetic rats. Afr J Tradit Complement Altern Med. 2009:6(1):30–41.
6. Okwu DE, Morah FNI. Antimicrobial and phytochemical evaluation of seed of *Garcina kola* and *Dennettia tripatala* fruits. Journal of medicinal and Aromatic Plant Science. 2006;4.
7. Chikezie PC, Agomuo EN, Amadi BA. Biochemistry practical/ research method, A fundamental approach. Megasoft Publishers Nigeria. 2008;(2):8-20.
8. Onwuka GI. Food analysis and instrumentation theory and practical. Naphthali Prints Nigeria. 2005;219.

9. Ekundayo EO, Ezeogu LI. Evaluation of antimicrobial activities of extracts of five plants used in traditional medicine in Nigeria. International J. of Tropical Medicine. 2006;93-96.
10. Anishmon VS, Toji T. *In vitro* antibacterial activity of *Lygodium flexuosum.* Nig Journal of Natural Products and Medicine. 2005;9:47.
11. Okigbo RN, Ogbonnaya UO. Antifungal effects of *Ocimum gratissimum* and *Aframomum melegeta* on post harvest yam (*Dioscorea spp*) rot. African Journal of Biotechnology. 2006;5(9):727-731.
12. Frantisek S. The natural guide to medicinal herbs and plants. Tiger Books International PLC, Twickenham, U.K. 1998;8:13-14.
13. Uchegbu RI, Okwu DE An evaluation of the phytochemical and nutrient composition of the seeds and stem bark of *Detarium senegalense* Gmeli. Journal of Natural Sciences Research. 2012;2(5): 107–111.
14. Okwu DE. Phytochemicals and vitamins content of indiginuous spices of south Easthern Nigeria. Journal of Sustainable Agriculture and Environment. 2004;6:30-37.
15. Salem K. How to discover abundant health and happiness. Morris Cerullo Mission to all the World. San Diego, California. 2000; 14.
16. Uchegbu RI, Mbadiugha CN, Njoku PC, Ekechi A. Comparison of the phytochemical and nutritional compositions of the seeds of *Mucuna flagellipes* and *Mucuna pruriens* (utilis). Alvana Journal of Science (Golden Jubilee Edition). 2014;7: 1-28.
17. Oduse KA, Idowu MA, Adegbite AFA. Chemical and phytochemical profile of some uncommon green leafy vegetables consumed in South West, Nigeria. IOSR Journal of Environmental Science, Toxicology and Food Technology. 2012; 1(3):22-26
18. Uchegbu RI, Akalazu JN, Ibe CO, Ahuchaogu AA, Amadikwa CU. Chemical composition of the stem extract of *Costus afer* (Bush Cane) and its antimicrobial activity. British Journal of Pharmaceutical Research. 2016;10(5):1-9.
19. Group E. Health effects of harmful organisms. Global Healing Center. 2010: 1-2.
20. Uchegbu RI, Mbadiugha CN, Ibe CO, Achinihu IO, Sokwaibe CE. Antioxidant, anti-inflammatory and antibacterial activities of the seeds of *Mucuna flagellipes*. American Journal of Chemistry and Applications. 2015;2(5):114-117.
21. Ukwubile CA, Henry N, Joshua VJ. Antimicrobial activity and characterization of *Annona muricata* leaf-loaded chitosan nanoparticles against cancer associated microbes. International Journal of Research Studies in Microbiology and Biotechnology. 2016;2(1):15-21.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18212