European Journal of Medicinal Plants
11(1): 1-6, 2016, Article no.EJMP.21502
ISSN: 2231-0894, NLM ID: 101583475

SCIENCEDOMAIN *international*
www.sciencedomain.org

Isolation and Characterization of Flavonoids in *Urena lobata* Leaves

Dixa Singh[1*] and V. S. Singh[1]

[1]Department of Chemistry, H.N.B. Garhwal University Campus-Pauri (Uttarakhand), India.

Authors' contributions

This work was carried out in collaboration by both authors. Author DS designed the study, performed the laboratory analysis and wrote the first draft of the manuscript. Author VSS helped and guided in the analysis and interpretation of the study and the literature searches. Both the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2016/21502
Editor(s):
(1) Marcello Iriti, Professor of Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Sapan Sharma, CSK Himachal Pradesh Agricultural University, India.
(2) Brahmadeo Dewprashad, City University of New York, USA.
(3) Bhaskar Sharma, Gyan Vihar University, Rajasthan, India.
Complete Peer review History: http://sciencedomain.org/review-history/11728

Original Research Article

Received 20th August 2015
Accepted 16th September 2015
Published 7th October 2015

ABSTRACT

Aim: *Urena lobata* L. a member of Malvaceae family, is widely used as famine food in Africa. It is also used in traditional medicine system to cure gonorrhea, fever, wounds, toothache & rheumatism. The plant has already been tested for its antioxidant activity. The work was extended to investigate and identify the flavonoid glycosides present in the plant.
Methodology: *Urena lobata* leaves were dried, powdered and extracted with petroleum ether followed by methanol. The methanolic extract, after processing through different solvents, was used to obtain the Chemically active constituents .Column Chromatography and Thin Layer Chromatography (TLC) were used to isolate four compounds which were, in turn, subjected to spectroscopic analysis.
Results: The spectroscopic studies indicated that the four isolated compounds are (1) Quercetin, (2) Kaempferol, (3) Quercetin 3-0-rutinoside and (4) Kaempferol 3-0- β glucopyranoside.
Conclusion: The presence of flavonoids in the plant makes it an important ingredient of the traditional medicinal system. Since flavonoids are associated with antioxidant activity, their presence in the plant makes it an important food material.

**Corresponding author: E-mail: dixa1974singh@gmail.com;*

Keywords: Urena lobata; flavonoids; quercetin; kaempferol; quercetin 3-0-rutin; kaempferol 3-0-β glucopyranoside.

1. INTRODUCTION

Flavonoids are a group of naturally occurring compounds having phenolic groups. These are also known as plant pigments and are found in all parts of a plant namely roots, stem .bark, leaves, flowers and fruits [1]. Flavonoids together with carotenes provide different colours to fruits, vegetables & herbs. The chemical structure of flavonoids is based on C_{15} skeleton with two benzene rings joined by three linear carbon atoms which may or may not be a part of third ring [2]. The skeleton of flavonoids consists of six major group namely flavone, chalcone, flavonol, flavonone, anthocyanin & iso-flavonone [3]. Flavonoids occur in the plants as aglycone (sugar free), glycosides (one or more sugar molecules attached to it) or methylated derivatives [4]. All flavonoids are water soluble. Flavonoids may acquire phenolic groups on 3, 5, 7, 2', 3', 4' & 5' positions in the skeleton. Flavonoids are secondary metabolites in plants. These are synthesized by phenyl -propenoid pathway. They are responsible for various biological and pharmacological activities in mammals [5], the most important being the antioxidant activity [5,6]. Beside, antioxidant activity, flavonoid show some other activities like microbial [7,8], antifungal, antiviral [8], anti-inflammatory [9], anticancer [10,11] and anti-diabetic [12].

Urena lobata L. plant is a member of the Malvaceae family and is commonly known as Bachita, Congo jute or Caesarweed [13]. It is a woody, erect perennial shrub reaching upto 2-3 m height. It is widely distributed in temperate and tropical zone of India. It is more or less hairy, the leaves varying in shape and size. The *Urena lobata* flowers are axillary, pink or purple of about 2.5 cm diameter.

Various extracts of leaves and roots of the plant are used in ancient medicine to treat diseases like diabetes, rheumatism, wounds, gonorrhea, tooth ache etc [14]. The leaves and flowers are eaten as famine food in Africa [15].

Earlier workers [16] have shown the presence of Mangeferin and Quercetin in aerial part of *Urena lobata*. Triglycerides were also isolated from the plant [17], imperatorin and furocoramin were isolated from the roots of the plant [18]. The authors have also determined presence of Quercetin and Kaempferol and examined anti - oxidant activity of the plant extract in their preceding work [6].

The work has been extended to ascertain the nature of bound flavonoids in the leaves of the plant extract.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant leaves were collected from Kotdwar region in Pauri District. The plant material was identified taxonomically by Dr. A. K. Agarwal, Associate Professor in Botany Department, Govt. P. G. College, Uttarkashi.

2.2 Extraction and Isolation

The plant leaves powder (2 Kg) was firstly extracted with petroleum ether (60℃- 80℃), then with methyl alcohol at 60℃ in a soxhlet apparatus for 36 hours. The methanolic extract was concentrated by distilling off the solvent. The methanolic residue (about 50 gm) was dissolved in water and then partitioned withn-hexane, chloroform and ethyl acetate respectively. The ethyl acetate fraction gave positive test for flavonoids [19]. Since the authors were interested in chemically active constituents, the fractions were subjected to chemical analysis. The ethyl acetate extract was subjected to column chromatography over silica gel. The column was eluted first with chloroform, then a chloroform – methanol mixture with increasing amount of methanol upto 20%. The fractions obtained from the column chromatography were subjected to TLC using $CHCl_3$ – CH_3OH (10:2) as the solvent system and examined under UV light alone and with ammonia vapours followed by locating reagent $FeCl_3$. The fractions eluted with 2 - 5% methyl alcohol were found to be the same after TLC analysis and therefore were combined. This combined fraction containing a mixture of three compounds, was again chromatographed on preparative silica gel TLC plates and eluted with $CHCl_3$: CH_3OH (10:2). Repeated purification of each compound by silica gel chromatography with methyl alcohol produced compound 1 (12.55 mg) and compound 2(14.20 mg). The fractions eluted with silica gel column chromatography with 6 - 8%

methyl alcohol, were found to be the same combined after TLC examination and so combined together. The major component found was purified by further column chromatography eluted with CH_3OH. This was named compound 3(8.20 mg). The fractions eluted with 10 -20% were combined as before after TLC analysis. The fraction was subjected to PTLC eluted with $CHCl_3$: CH_3OH (10:2) to give one major spot. Further purified on silica gel column chromatography eluted with methyl alcohol to give pure compound 4 (9.82 mg).

3. RESULTS AND DISCUSSION

The four compounds obtained, as mentioned in the experimental section, were subjected to spectroscopic analysis for identification. The Techniques used were UV, IR, ^{1}HNMR & ^{13}CNMR spectroscopy. The details of the spectral peaks were noted as follows.

Compound (1)

Amorphous yellow powder UV λmax nm (MeOH): 254 sh, 268, 322 sh,365;+NaOMe : 275, 320, 416; +$AlCl_3$: 262 sh, 270, 352, 426; +$AlCl_3$ / HCl: 260, 271, 350, 426; +NaOAc: 275, 300, 385; +NaOAc /H_3BO_3:269, 295sh,320sh,370. ^{1}HNMR (200 MHz, DMSO-d_6) δ: 8.00 (2H, d, J =8.1 Hz,H-3', H-5'),6.90 (2H,d,J=8.1Hz, H-2', H-6'),6.40 (1H,d J=2.5 Hz, H-8), 6.21 (1H,d, J=2.5Hz, H-6).^{13}CNMR (CD_3OD): δ:94.2(C-8), 99.0 (C-6), 104.1(C-10),115.9 (C-3', C-5'), 123.4 (C-1'),130.4 (C-2',C-6'),136.9 (C-3), 147.8 (C-9),158 (C-2),160.4(C-5),161.8 (C-7),165.4(C-4')& 177.2(C-4).

Compound (2):

Yellow crystal, UV λmax nm (MeOH): 258, 266sh, 299 sh, 360; + NaOMe: 272,327,416; +$AlCl_3$: 275, 303 sh,430; +$AlCl_3$ / HCl: 271,300, 364sh, 402; +NaOAc: 270, 325 ,393; + NaOAc / H_3BO_3: 262,298,387. ^{1}HNMR (200 MHz, DMSO-d_6) δ:7.28 (dd,J= 1.4 H-6') &.7.51 (d J=1.4, H-2'), 6.88(1H,d,J=8.0Hz, H-5'),6.40 (1H,d,J=2.5Hz, H-8),620 (1H,d,J=2.5Hz, H-6).

Compound (3)

Yellow needle. UV λmax nm (MeOH): 254, 350; +NaOAc: 275, 306, 366; +NaOAc / H_3BO_3: 268, 351; +NaOMe: 276,327,404; +$AlCl_3$: 265, 305,352; +$AlCl_3$ /HCl: 272,(300), 400nm.IR (KBr): 3240,1710, 1660, 1182 - 1081 & 832 cm^{-1}. ^{1}H NMR (300 MHz, CD_3 OD) δ: 11.92 (OH -5), 10.86 (7- OH), 6.68 (H-8,d,j=2.1hz), 6.50 (H-6,d,j=2.8hz), 7.85 (H-2',d,J=8.5hz), 6.94 (H-3',d,j=8.9), 9.54 (4'-OH), 6.94 (H -5',d,J=8.9), 7.85(H-6',d,j=8.5), 4.64(H-1'',d,j=7.8),4.07 (H-2'',dd,j=9.6),3.77(H-3'',d, j =9.6),3.48 (H-4'',t,j=8.9),3.68(H-5'',d,j=9.6,2.6hz) & 4.55(H-6'', dd, J=10,2.6hz).
C^{13}NMR (75MHz,CD_3OD),δ:156.91(C-2),135.51(C-3),175.90(C-4),165.53(C-5), 99.52(C-6), 160.0(C-7), 91.80 (C-8),105.13(C-9),152.52(C-10),121.82(C-1'),130.10(C-2'), 115.41(C-3'),157.53(C-4'),115.42 (C-5'), 130.10 (C-6'),SUGAR MOIETY- δ:101.42(C-1''),74(C-2''),78.51(C-3''),71.31(C-4''),78.40(C-5'') & 62.32(C-6'').

Compound (4)

Amorphous yellow powder, UV λmax nm (MeOH): 255, 352 IR (KBr): 3260, 1660.85, 1606.85, 1504.61,1454,1359,1304,1197,1060,1010,933, 798, 709, 644,592 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 6.16 (1H,d,J=2.2,H-6), 6.35 (1H,d,J=2.2,H-8),7.35 (1H,d,J=1.8,H-2'),6.81 (1H,d,J=8,H-5'),7.50(1H,dd,J=8.0,H-6'), 5.32 (1H,d,J=7.4,H-1''), 3.01-3.17 (4H,m,H-2'',H-3'',H-4'',H-5''), 3.30 (1H,m,Ha -6''), 3.68 (1H, d, J=10.3 Hz, Hb -6''), 4.36 (1H, d,J=1.8,H-1'''), 3.01 - 3.07 (4H, m, H-2''', H-3''', H -4''' & H -5'''), 0.97 (3H,d,J=6.2Hz, CH_3- 6'''),12.59 (1H,S,5-OH), 9.39 (1H,s,OH-5'),9.45 (1H,s,OH-4')&10.73 (1H,s, OH-7). C^{13}NMR: (125 MHz, DMSO - d_6) δ: 177.40 (C-4), 164.25 (C-7),161.25 (C-5),156.52 (C-2) 156.48 (C-9), 133.34 (C-3), 104.12(C-10),121.3 (C-1'),148.53 (C-4'), 144.8(C-3'), 121.33(C-6'), 115.36 (C-2'),116.38 (C-5'),104.12 (C-10), 98.84 (C-6),Glucose moiety δ:101.79 (C-1''),74.42 (C-2''), 77.12 (C-3''), 70.48(C-4''), 77.05(C-5''),60.85(C-6''), Rhamnose moiety δ:100.72 (C-1'''),70.48(C-2'''), 70.48 (C-3'''),72.17(C-4'''),68.30(C-5''') & 17.52 (C-6''').

The interpretation of the spectral peaks may be as follows.

Compound (1):

The proton NMR spectrum of the compound produced four proton doublet peaks at the aromatic region. The aromatic proton doublets at δ 6.21 and δ 6.40 were due to the meta coupled protons of a 5,7 substituted ring A. This accounted for protons H-6 & H-8 respectively. The two proton doublets at δ 6.90 & δ8.00 accounted for the protons H-2',H-6' and H-3',H-5'

of a 4' –substituted ring B respectively. The spectral data were in agreement with Kaempferol reported in the literature [20]. Thus compound (1) is identified as Kaempferol (Fig. 1).

Compound (2):

The proton NMR spectrum showed five aromatic signals. The aromatic proton doublet at δ6 .20 & δ6.40 were due to the meta coupled protons of a 5,7 substituted ring A, for protons H-6 & H-8 respectively. The peaks at δ6.88, δ7.28 & 7.51 were assigned for protons H-5', H-6' & H-2' showing an ABX coupling system of a 3', 4' substituted ring B of a flavonol. The structure of this compound was confirmed (Fig. 2) by comparing ^{1}HNMR of Quercetin from the literature [21].

Compound (3):

The IR (KBr) spectrum showed that the absorption band at 3240cm^{-1} is the stretching vibration of hydroxyl group (OH), 1710cm^{-1} is due to C=O stretching vibration ,1660cm^{-1} is the stretching vibration of C=C bond of aromatic ring,1450-1356 cm^{-1} is bending vibration of C-H bond, 1182-1081 cm^{-1}is due to the presence of C-O-C bond of ether ,832cm^{-1}is the C-H bond outside the field. ^{1}HNMR (300MHz,CD_3OD) indicates that the compound is a group of flavonoid glycosides. 3 sharp peaks as singlet δ 11.92, 10.86 & 9.54 shows that there are three hydroxyl group present at 5-OH, 7-OH & 4'OH respectively. The presence of aromatic proton at δ 7.85(2H,d, ,J=8.5Hz, H-2' & H-6') & δ 6.94 (2H,d, J=8.9Hz for H-3' & H-5').Besides, the two aromatic proton as singlet at δ 6.68 (1H,s) for H-8 and δ 6.50 (1H,s) for H-6. C^{13}NMR (75MHz,CD_3OD) showed that compound has 21 carbon atoms in which, there were nine quaternary carbon (C-2,C-3,C-4,C-5,C-7,C-9,C-10,C-1' & C-4') and one CH_2 group of sugar at δ 62.32 7 & five methyne group for sugar at C-1'', C-2'', C-3'', C-4'' & C-5''. Remaining four would be C-6 C-8, C-2' C-3',C-5' C-6'. The HMBC and HMQC spectra showed an aromatic proton at δ 6.68 (at C-8) correlates to carbon signal at 95.3 (atC-6), correlates to carbon (at C-10,C-9,C-6 & C-7). The correlation between proton at δ 6.50 (at C-6) to carbon signal at 103.9 (at C-9), correlated to the C at C-5, C-10, C-7 & C-8. The aromatic proton at δ 6.94 correlates to carbon signal at C-3 & C-5') and at C-4' & C-2''.Glucoside proton present at C-1'''at δ 4.64 correlates to carbon signal at C-1'', also correlates to the C at C-2'',C-3'',C-1,C-2 & C-3.Similarly, proton of C-2''at δ 4.07 correlates to the carbon signal at C-2'',correlates to the C at C-3''. The correlation between C-3' at δ 3.77 to carbon signal at C-3'', correlate to the C at C-4'' & H of the C-4''at δ 3.48 correlates to the Carbon signal at C-4'', also correlates to C at C-3'' & H of C-5 at δ3.68 correlates with C-5'',C- 4'' & C-3''. On the basis of HMBC spectra identified that a correlation between the anomeric proton present at C-1''' with C-1,C-2& C-3 indicate that the group is located at the C-6 position. On the basis of UV, IR, ^{1}HNMR, C^{13}NMR and HMBC data analysis the compound 3 is identified as Kaempferol 3-O–beta glucopyranoside (Fig. 3).

Compound (4):

The UV spectrum showed absorption band at 255 & 352 nm for 3-substituted flavanol. IR spectrum showed a strong absorption peak at 1660cm^{-1} for a carbonyl group & intense broad band at 3260 cm^{-1} due to OH group. A peak at 1606 cm^{-1} due to stretching vibration of C =C bond of aromatic ring at 1450-1356 cm^{-1} due to bending vibration of CH bond. The aromatic region of ^{1}HNMR spectrum of the compound has the characteristic of Quercetin moiety. ^{1}HNMR signals at δ 6.16 (1H,s),6.35 (1H,s) are due to meta coupled protons of ring A (H-6 & H-8) of a flavonoid nucleus. It clearly indicates that the compound must be 5,7 dihydroxy flavonol. A signal at δ 7.53 (d,J=1.8),6.81 (d,J=8), 7.50 (d, J=8) are assigned to H-2', H-5' & H-6' of ring B respectively of Quercetin nucleus. The ^{13}C NMR spectrum showed signals which indicated the presence of flavonoids moiety along with two different sugar moieties & these two sugar moieties were confirmed as β glucose and α rhaminose by the spectroscopic analysis of ^{1}HNMR & ^{13}C NMR data. The ^{1}HNMR spectra showed a singlet peak at δ 5.08 ppm attributed to the anomeric α rhamnopyranosyl proton & a doublet at δ 5.50 (J=7.4 hz) with diaxial coupling assignable to the anomeric β glucopyanosyl proton with chemical shift characteristics of glycosidation in a close position i.e.in C-2 position of glycosyl residue (Δδ -0.5 ppm) downfield shift in comparing with chemical shift of anomeric proton signal in quercetin 3-glucoside (2.22). In ^{13}C NMR of compound the C-1 & C-2 carbon signal of the glucosyl moiety were observed at 98.97 and 77.42 ppm respectively which proved that the suggested interglycosidic bond (1→2) by recongnisable downfield shift of C-2 carbon signal (Δδ -3.3 ppm) upfield shift of the C-1 signal (Δδ -2.1 ppm) in comparison with quercetin 3 glucoside (2.23).

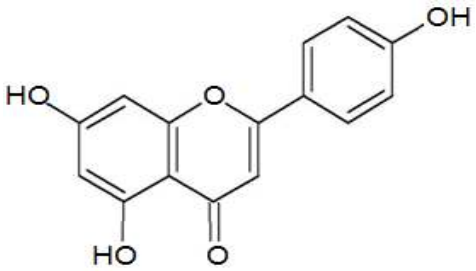

Fig. 1. Kaempferol

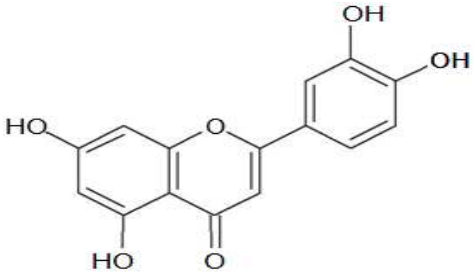

Fig. 2. Quercetin

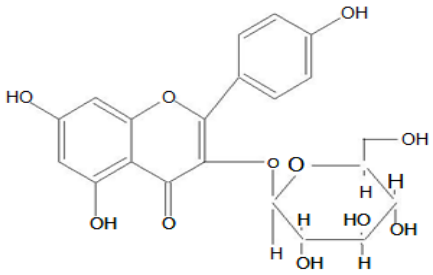

Fig. 3. Kaempferol 3 –O-beta glucopyranoside

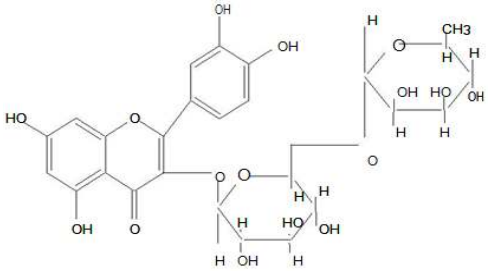

Fig. 4. Quercetin 3-O- rutinoside

^{13}C NMR showed that the compound containing 28 carbon atoms consisting one CH_2 group at δ 60.85 and one CH_3 group at δ 17.52. Downfield shift of C-6'' in ^{13}C NMR spectra & HMBC correlation between H-1''' (δ 4.36) & C-6'' (δ 60.85) indicated that rhamnose was linked to the C-6'' of glucose, confirming the rutinose unit as a sugar moiety. The attachment of rutinose moiety to the aglycone was determined from the correlation between H-1''(δ 5.32,d) & C-3 (δ 133) of aglycone. On the basis of UV, IR, ^{1}HNMR, ^{13}C NMR, HMBC data analysis the compound 4 is identified as Quercertin 3-O–rutinoside (see above Fig. 4).

4. CONCLUSION

It has been established that *Urena lobata* leaves contain four important flavonoids, two in aglycone and two in glycoside form. The leaves can serve as good food supplement due to presence of the flavonoids. Since there are four types of flavonoids present in the leaves, these can work as a good antioxidant.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tsukasa I, The structure & distribution of flavonoids in plants. J of Plant Research. 2000;113:287-299.
2. Middleton E, The flavonoids. Trends in Pharmacological Sciences. 1984;5:335-338.
3. Narayana KR, Reddy MS, Chauvedi MR, Krishna DR. Biflavonoids, classification, pharmacological & biochemical effects & therapeutic potential. Indian J of Pharmacology. 2001;33:2-16.
4. Harbone JB. The flavonoids. Advances in Research Sciences, 1980.ed.1.London: Chapman & Hall; 1998.
5. Robak J, Gryglewski RJ, Biocativity of flavonoids. Pol J Pharmacol. 1996;48:555-564.
6. Singh D, Singh VS. *Urena lobata*: A green source of antioxidant. J of Plant Sciences. 2014;2(6):299-303.
7. Cushine TP, Lamb AJ, Antimicrobial activity of flavonoids. International J of Antimicrobial Agents. 2005;26(5):343-356.

8. Friedman M, Overview of antimicrobial, antitoxin, antiviral & antifungal activities of the flavonoids & tea. Molecular Nutrition & Food Research. 2007;51(1):116-134.
9. Murlidhar A, Babu KS, Sankar TR, Redenna P, Reddy GV, Latha J. Antiflammatory activity of flavonoid fraction isolated from stem bark of *Butea monosperma* (Lam): A mechanism based study. International J of Phyto-pharmacology. 2010;1:124-132.
10. Davis WL, Mathew SB. Antioxiodants and Cancer III: Quercetin. Alternative Medicine Review. 2000;5(3):196-208.
11. Romagnolo DF, Selmini OI. Flavonoid & Cancer prevention: A review of the evidences. J Nutr Gerontol Geriatr. 2012;31(3):206-238.
12. Josline YS, Halana NM. Antidiabetic properties of flavonoid compound isolated from *Hyphaena thebaica* epicarp on alloxan induced diabetic rats, Pharmacognosy Research. 2013;5(1):22-29.
13. Carl Linnaeus, Species Plantarum, Laurentius Salvius. 1753;2:692.
14. Francis JK. Atlas of florida vascular plants, Institute of systematic Botany, University of South Florida, Tampa, Fl; 2003.
15. Freedman R. Famine foods: Malvaceae, Predue University, Perdue, IN; 1998.
16. Srinivasan KK, Subramanian SS. Isolation of Mangiferin from *Urena lobata*. Arog J Health Sci. 1981;7(1):140-141.
17. Morelli CF, Cairoli P, Speranza GM, Rajia S. Triglycerides from *U.lobata.* Fitoterapia. 2006;77(4):296-299.
18. Keshab G. A Furocouramin, Imperatoin isolated from *Urena lobata.* Molebank 2004;M382.
19. Chatwal GR. Organic chemistry of natural product. 4ed. Mumbai: Himalaya Publication House; 2007.
20. Markham KR, Ternal B, Stanley R, Geiger H, Mabry TJ. C13 NMR studies of flavonoids-III: Naturally ouccuring flavonoids glycosides and their acylated derivatives. Tetrahedron. 1978;34:1389-1397.
21. Wollenweber E, Dietz VH, Occurrence and distribution of free flavonoid aglycones in plants. Phytochem. 1981;20(5):859–932.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11728