INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

ISOLATION AND CHARACTERIZATION OF NATURAL MUCILAGE FROM *LAGENARIA SICERARIA*

Balagani Pavan Kumar[1]*, Manubolu Sindhuri[3], K. Jyothshna Devi[1], S. Vinoth Kumar[2], Alluru Manogna[3], Pichika Madhavi[3]
[1]Assistant Professor, Department of Pharmaceutics, Gokula Krishna College of Pharmacy, Sullurpet, Nellore Dist, A.P, India
[2]Assistant Professor, Department of Pharmaceutical Chemistry, Gokula Krishna College of Pharmacy, Sullurpet, Nellore Dist, A.P, India
[3]Gokula Krishna College of Pharmacy, Sullurpet, Nellore Dist, A.P, India
*Corresponding Author Email: pavan.gkcp@rediffmail.com

Article Received on: 10/10/13 Revised on: 29/10/13 Approved for publication: 17/11/13

DOI: 10.7897/2230-8407.041126
IRJP is an official publication of Moksha Publishing House. Website: www.mokshaph.com

ABSTRACT
Development of new excipients is time consuming, involves tedious procedures and is highly expensive. Instead, identification of new uses for the existing substances is relatively inexpensive and less time consuming. The intension of the present study was designed for isolation and characterization of mucilage from the pulp of *Lagenaria siceraria* and explores its use as pharmaceutical excipients. Various methods to isolate mucilage were tried and simple, economical and optimum method was developed. Physicochemical properties of *Lagenaria siceraria* such as appearance, odour, solubility, Bulk and Tapped Densities, Hausner's Ratio, swelling index, pH, compressibility and Angle of Repose were studied. The average yield of dried mucilage obtained from *Lagenaria siceraria* pulp mucilage was 20 g/kg. Bulk density was found to be 0.256 g/cm^3 and tapped density was 0.35 g/cm^3, Carr's index was 19.1 %, Hausner ratio was 1.306 and angle of repose was found to be 20^0.29". Phytochemical tests carried out on *Lagenaria siceraria* pulp mucilage confirmed the presence of vitamins, glycosides and carbohydrates. The FTIR spectrum of *Lagenaria siceraria* pulp mucilage showed N-H stretching, O-H stretching, C-H stretching, C=O stretching C=N stretching and C-C stretching at respective wave lengths. DSC thermogram of pulp powder shows sharp endothermic peak at 87.05°C and exothermic peak at 333.23°C. Scanning Electron Microscopy of *Lagenaria siceraria* pulp shows that they have irregular size and shapes. From the Study it is very clear that, *Lagenaria siceraria* pulp extract has wide range of applications and ensures as a promising excipient in various pharmaceutical formulations.
Keywords: *Lagenaria siceraria*, calabash, mucilage, polymer, Natural excipient.

INTRODUCTION

Excipients are inert molecules that play a very important role in designing of dosage form[1]. Today, we have a number of plant-based pharmaceutical excipients, which may be selected and optimised based on the properties of drug, requirements of the dosage form and its site of action. Apart from its common functions like serving as inert vehicle for the administration of right volume of active pharmaceutical ingredient with consistency in weight, excipients also fulfil multifunctional roles such as release retardants, solubility enhancers, viscosity modifiers etc. In addition to this they offer significant advantages in ease of manufacturing, enhancement of patient compliance, improved bioavailability, reproducibility, targeted delivery etc[2]. Majority of investigations on natural polymers in drug delivery systems are centred because natural ingredients, either active or inactive, are in high demand for their drug delivery applications because of their versatile availability, low cost as compared to synthetic and semi-synthetic products and their biocompatible and biodegradable nature[3]. Hence in this study we selected *Lagenaria siceraria* for the isolation of natural mucilage from the fruit pulp. *Lagenaria siceraria* fruit is popularly known as calabash or bottle gourd in India[4]. The bottle gourd is generally available in the warmer region of the world. Bottle gourd belongs to genus lagenaria is derived from the word lagena means bottle[5]. It is having various pharmacological activities. Various literature reports revealed that the pharmacological properties of lagenaria[6]. Its common names include calabash, bottle gourd (Eng.); Sorakaya, anpakaya (Telugu); alabu, tumbi in ancient days, Iksuaku, katutumbi and mahaphala in recent days (Sanskrit); lauki or ghia (Hindi); dudhi or tumbadi (Gujarati); sorakkai (Tamil); chorakkaurdu (Malayalam); Sorakay, tumbi (Kannada); Lausag, lokitumbi (Bengali) and ghiya (Urdu). Geographically it occurs throughout India and is now cultivated worldwide[7]. The fruit is traditionally used as cardiotonic, aphrodisiac and general tonic[8], liver tonic and against liver disorders, anti-inflammatory, expectorant and diuretic agent[9]. Further anti hepatotoxicity effect of fruit pulp[10], analgesic and anti-inflammatory activity of fruit juice[11] and hypolipidemic activity of fruit has been evaluated[12]. Recently, the anti-oxidant activity of ethanolic extract of epicarp and fruit juice of *Lagenaria siceraria* fruit has been evaluated[13]. The objective of the work is to isolate mucilage from the pulp of *Lagenaria siceraria* and explore its use as a pharmaceutical excipient. Various methods to isolate polymer were tried and simple, economical and optimum method was selected. After isolation of the mucilage it is subjected to flow properties, spectral analysis, thermal analysis and morphological studies.

MATERIALS AND METHODS

Isolation of mucilage from *Lagenaria siceraria* pulp

Mucilage can be isolated by different methods. A number of methods were used to isolate the mucilage from *Lagenaria siceraria* pulp. The mucilage isolated from the different methods was compared for the yield of the mucilage. The procedure giving maximum yield of mucilage was used for isolation. The various isolation methods used to isolate the *Lagenaria siceraria* pulp mucilage are given below.

- General methods of mucilage Isolation

a) Precipitation of mucilage in alcohol
Boiled extract of pulp was precipitated in alcohol.

b) Precipitation of mucilage in acetone
Boiled extract of soaked and crushed pulp was precipitated in acetone

- Microwave oven extraction
- By deflating with petroleum ether

a. Deflating by 12 hrs and occasional shaking with petroleum ether.

b. Deflating by Soxhlet apparatus with petroleum Ether.

General Methods of Mucilage Isolation

Two general methods were followed for the isolation of mucilage from *Lagenaria siceraria* pulp[18].

Precipitation of Mucilage in Alcohol

Boiled extract of mucilage was precipitated in alcohol

100 g of *Lagenaria siceraria* pulp were taken in a beaker and 1 litre of distilled water was added to it. Then it was boiled for 15 minutes. and was filtered by Buchner funnel without filter paper. The residue was again boiled with 500 ml of distilled water and filtered. The combined filtrate was then passed through muslin cloth with 8 folds. The filtrate was then precipitated in ethanol and then dried in the oven. The yield was recorded.

Precipitation of Mucilage in Acetone

Boiled extract of soaked and crushed pulp was precipitated in acetone

100 g of *Lagenaria siceraria* pulp is boiled in 1 litre of distilled water. The boiled pulp mass was then filtered by the muslin cloth with 8 folds. The filtrate was then precipitated using 1000 ml of acetone. The precipitated mucilage was separated and dried in the oven. The weight of obtained mucilage was recorded.

Microwave oven extraction method

Lagenaria siceraria pulp was blended in the mechanical blender for 5 minutes and soaked in the distilled water (150 ml) for 24 h in 1000 ml beaker. It was kept in the microwave oven along with the glass tube inside to prevent bumping. It was subjected to microwave irradiation at 800W intensity for 3 minutes. The beaker was removed and kept aside for 2 h for the release of mucilage into the water. The material was filter through the muslin bag and hot water (25 ml) was added through the sides of the marc and squeezed well in order to remove the mucilage completely. The equal amount of ethanol was added to the filtrate to precipitate the mucilage and kept inside a refrigerator for one day for effective settling. It was filtered and dried completely in an incubator at 37°C[14].

By deflating with petroleum ether

Deflating by 12 h shaking with petroleum ether

The *Lagenaria siceraria* pulp was blended and kept in contact with petroleum ether in a conical flask for 12 h. The flask was kept on the electrical shaker for the continuous shaking. The material was then filtered out and dried at room temperature for complete removal of petroleum ether. The blended powder was then soaked in distilled water. The swollen wet mass was then spread on a glass tray and dried at 60°C. The dried material was then passed through mesh #30. The material was winnowed and again passed through mesh #60. The weight of mucilage obtained was recorded[15].

Deflating by Soxhlet apparatus with petroleum ether

The *Lagenaria siceraria* pulp was blended and kept in contact with petroleum ether in Soxhlet apparatus. The cycles of petroleum ether were run till complete deflating was obtained. The defatted material was then dried at room temperature for complete removal of petroleum ether. The dried defatted seed powder was then soaked in distilled water. The swollen wet mass was then spread on the glass tray and dried at 60°C. The dried material was then passed through mesh #30. The material was winnowed and again passed through mesh #60. The weight of mucilage obtained was recorded. The method yielding maximum amount of mucilage was utilized further to obtained required quantity of mucilage and used in further studies.

Characterization of mucilage

Physical properties characterization

The dried mucilage was studied for percentage yield, appearance, solubility, pH, swelling index[16].

Chemical characterization

The mucilage collected from pulp was tested for chemical characteristics for identification i.e. test for carbohydrates, test for tannins, test for alkaloids, flavanoids, steroids, amino acids, terpins, saponins, oils, fats, Phenols and resins. The results were tabulated[16].

pH of solution

The pH of the 1 % w/v aqueous mucilage solution was measured with a pH meter[17] (Equip-Tronics, EQ-610).

Determination of swelling index

Swelling characteristics of the mucilage was tested in distilled water. The Swelling index is the volume in ml occupied by 1 g of the substance. The Swelling index of the mucilage powder was determined according to British Pharmacopoeia method 6. The test was performed by taking 1 g of the mucilage powder in a 50.0 ml ground glass stoppered cylinder graduated over a height of 120 to 130 mm in 0.5 divisions. To this 25 ml of distilled water was added and this was shaken vigorously every 10 minutes for 1 hour and then allowed to stand for 24 hours. The volume occupied by the mucilage powder was measured[18].

Flow properties

The dried pulp mucilage powder was examined for flow properties like angle of repose, bulk density, tapped density, Carr's index, compressibility index and hausner's ratio[19-21].

Spectral Studies by FTIR

The Fourier transform infra-red analysis was conducted for the structure characterization. FTIR spectra of the pulp powder were recorded. Air dried powder were taken in a KBr pellet using BOMEN MB SERIES FTIR instrument. Approximately 5 mg of samples were mixed with 50 mg of spectroscopic grade KBr; samples were scanned in the IR range from 500 to 4000 cm^{-1}.

Thermal Studies by DSC

Weighed amount of sample were placed in hermitically sealed aluminium pans and were heated at a speed of 20°C/min over a temperature range of 50°C to 450°C in a differential scanning calorimetry (Perkin-elmer DSC-7) at a chart speed of 10 mm/min.

Morphological studies by SEM

Scanning Electron Microscopy is an excellent tool for physical observation and morphological features. For the external morphology studies, air dried pulp was visualized using scanning electron microscopy samples were dusted on a double sided adhesive tape. Excess samples removed and is coated with 30 nm layer of gold using JEOL JFC 1100E splitter coater. The coated samples were viewed under a scanning electron microscope and scanning electron

micrographs were taken by using JOEL – JFC 5300 scanning microscope.

Table 1: Comparison of mucilage's isolated by different methods

S. No	Method of Isolation	% Yield
1	Boiled extract of seeds precipitated using alcohol	9
2	Boiled extract of soaked and crushed seeds precipitated using alcohol	11
3	Boiled extract of soaked and crushed seeds precipitated using acetone	5
4	Precipitation of soaked and blended seeds using acetone	6
5	Microwave oven extraction	8
6	Defatting by 12 h. shaking with petroleum Ether	46
7	Defatting by Soxhlet apparatus with petroleum Ether	45

Table 2: Physical Characterization of *Lagenaria siceraria* pulp mucilage

S. No.	Physical Properties	Observation
1	Appearance	Light cream colour
2	Odour	Characteristic
3	Solubility	Soluble in carbon tetra chloride and Slightly soluble in chloroform
4	Swelling index	19-20
5	pH (1 % w/v)	6.4

Table 3: Chemical Characterization of *Lagenaria siceraria* pulp mucilage

S. No	Constituent	Observation
1	Alkaloids	_
2	Carbohydrates	+
3	Glycosides	+
4	Saponins	-
5	Tannins	-
6	Vitamins	+
7	Lipids	+
8	Gums and mucilage's	+

Table 4: Micromeritic properties of *Lagenaria siceraria* pulp mucilage

S. No	Flow Property	Results
1	Bulk density	0.256 g/cm^3
2	Tapped density	0.35 g/cm^3
3	Compressibility	20.29
4	Angle of repose	20^0.30"
5	Carr's Index (%)	19.1
6	Hausner ratio	1.306

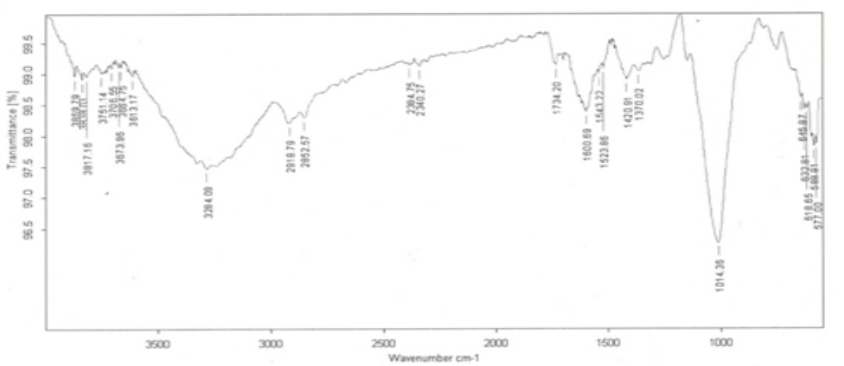

Figure 1: FTIR Spectra of *Lagenaria siceraria* Pulp mucilage

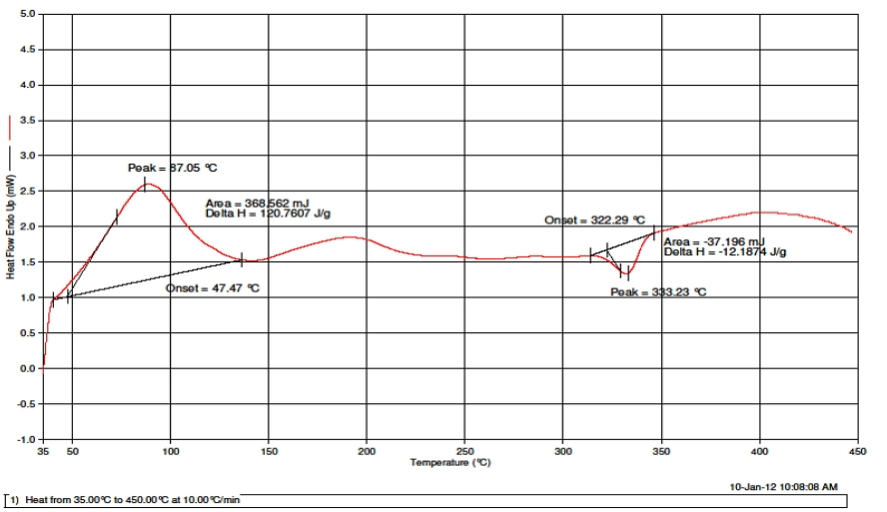

Figure 2: DSC thermogram of *Lagenaria siceraria* pulp mucilage

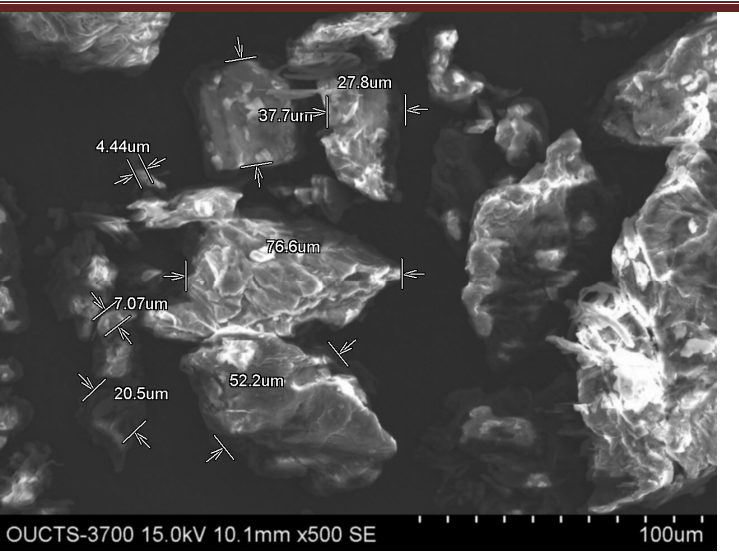

Figure 3: SEM photographs of *Lagenaria siceraria* pulp mucilage

RESULTS AND DISCUSSION

Lagenaria siceraria pulp mucilage was isolated by different methods like precipitation and defatting. But, the defatting of isolation with petroleum ether using Soxhlet apparatus shown the highest % yield of 46% when compared to other methods and the details were shown in Table 1. The average yield of dried mucilage obtained from *Lagenaria siceraria* pulp mucilage was 20 g/kg. The mucilage obtained was subjected to physicochemical characteristics and the results were summarized in Table 2. The mucilage is soluble in carbon tetrachloride and slightly soluble in chloroform. Bulk density was found to be 0.256 g/cm^3 and tapped density was 0.35 g/cm^3, Carr's index was 19.1 %, Hausner ratio was 1.306 and angle of repose was found to be 20^0.29". Results obtained were within the limits and indicated good flow properties. The flow properties are shown in table 4. Phytochemical tests carried out on *Lagenaria siceraria* pulp mucilage confirmed the presence of vitamins, glycosides and carbohydrates. On treatment of mucilage with ruthenium red, it showed red colour confirming the obtained product as mucilage. A violet ring was formed at the junction of two liquids on reaction with Molisch's reagent indicating the presence of carbohydrates. The results of Phytochemical screening of mucilage are summarized in Table 3. The FTIR spectrum of *Lagenaria siceraria* pulp mucilage showed sharp and characteristic peaks at 3673.95, 3284.06, 2918.79, 1734.20, 1600.09 and 1014.35 cm^{-1} which corresponds to N-H stretching, O-H stretching, C-H stretching, C=O stretching C=N stretching and C-C stretching respectively. The FTIR spectra's were shown in shown in Figure 1. DSC is a very useful tool in the investigation of thermal properties of a compound. DSC thermogram of pulp powder shows sharp endothermic peak at 87.05°C and exothermic peak at 333.23^{O}C. DSC thermograms were shown in Figure 2. Scanning Electron Microscopy of *Lagenaria siceraria* pulp shows that they have irregular size and shapes. The average particle size of pulp ranges from 4.44 to 76.6 µm. SEM photographs was shown in Figure 3.

CONCLUSION

From this investigation, we conclude that *Lagenaria siceraria* pulp extract has wide range of applications and ensures as a promising excipient in various pharmaceutical formulations. The method employed for isolation of polymer i.e., with acetone is simple, economic and gives maximum total yield as compared to other methods of isolation. However further study is essential to determine its category as excipient in dosage forms.

ACKNOWLEDGMENT

The authors are thankful to Shri C. Srinivasa Baba, secretary and Dr. I. Sarath Chandiran Principal of Gokula Krishna College of Pharmacy, Sullurpet, SPSR Nellore Dist, A.P, India for availing the laboratory facilities during the course of research studies.

REFERENCES

1. Anonymous. http://www.greenpharmacy.info/temp/Int J Green pharm64270-8387902-231759.pdf. gums, mucilage's and resins in novel drug delivery systems. Ind J pharm Edu Res 2011; 45: 86-99.
2. Anonymous. http://goldbook.iupac.org/M03667.html. Mc Crum NG, Buckley CP, Bucknall CB. Principles of polymer engineering. Oxford; New York: Oxford University Press; 1997. p. 1.
3. Anonymous. The Ayurvedic Pharmacopoeia of India, Part 1. 1st ed. Vol. 1. New Delhi Civil lines, Delhi: The controller Of India, Government of India Ministry of Health and family Welfare Development of Indian System of Medicine and Homoeopathy; 2001. p. 215-6.
4. Prajapati Rakesh P, Kalariya Manisha, Parmar Sachin K, Sheth Navin R. Phytochemical and pharmacological review of *Lagenaria siceraria*. J Ayurveda Integr Med 2010; 1: 266-272. http://dx.doi.org/10.4103/0975-9476.74431 PMid:21731373 PMCid:PMC3117318
5. Shah BN, Seth AK and Desai RV. Phytopharmacological Profile of *Lagenaria siceraria*: A Review. Asian J. Plant Sci 2010; 9(3): 152-157. http://dx.doi.org/10.3923/ajps.2010.152.157
6. T Sivannarayana, SK Saddam Hussain, K Phani Jithendra. Pharmacological and pharmaceutical applications of *Lagenaria siceraria.* Int. J. Inv. Pharm. Sci 2013; 1(4): 288-292.
7. www.plantzafrica.com Mienkie Welman National Herbarium, Pretoria; 2005.
8. Vaidhya LS. Shaligram Nighantu Bhushanam, Khemraj Srikrishnadas, Steam press, Mumbai; 1953. p. 891-2.
9. Warrior PK, Nambiar VPK and Ramakutty C. *Lagenaria siceraria* (Mol) Standley, in Indian Medicinal Plants 1995; 3: 292-5.
10. Shirwaikar K and Srinivasan KK. Chemical investigation and anti-hepatotoxicity activity of the fruits of *Lagenaria siceraria* Indian J Pharm Sci 1996; 58: 197.
11. BV Ghule, MH Ghante, AB Upaganlawar, PG Yeole. Analgesic and Anti-Inflammatory activities of *Lagenaria siceraria* Stand. fruit juice extract in rats and mice, Pharmacognosy Magazine 2006; 2(8): 232-238.
12. Ghule BV, Ghante MH, Saoji AN, Yeole PG. Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol.) fruit extracts. Indian J Exp Biol 2006; 44(11): 905-9. PMid:17205712
13. Deshpande JR, Mishra MR, Meghre VS, Wadhodkar SG and Dorle AK. Free Radical Scavenging activity of *Lagenaria siceraria* (Mol) Standley fruit, Natural Prod Rad 2007; 6(2): 127-300.

14. Shah BN, Seth AK. Microwave assisted isolation of mucilage from the fruits of *Abelmoschus esculentus*. Hygeia journal for drugs and medicines 2011; 3(1): 54- 57.
15. Sharma VK, Tiwari M, Chauhan NS, Nema RK. Phytochemical investigation on the Ethanolic Extract on the Leaves of *Zizyphus xylopyrus* (Retz.) Willd. International journal of Agronomy and Plant Production 2012; 3(1): 26-37.
16. Indian Pharmacopoeia 1996; 2: 446-447.
17. Fedeniuk RW, Biliaderis CG. Composition and Physicochemical Properties of Linseed (*Linum usitatissimum* L.) Mucilage. J. Agric. Food Chem 1994; 42(2): 240-247. http://dx.doi.org/10.1021/jf00038a003
18. Trishna Bal, Padala Narasimha Murthy, Shubhranshu Sengupta. Isolation and analytical studies of mucilage obtained from the seeds of *Dillenia indica* by use of various analytical techniques. Asian journal of pharmaceutical and clinical research 2012; 5(3): 65.
19. Pabon CV, Frutos P, Lastres JL, Frutos G. *In-vitro* study of mixed controlled release matrix tablets containing HPMC and polyamide. Drug Develop Ind Pharm 1992; 18(20): 2163-2171. http://dx.doi .org/10.3109/03639049209038754
20. Efentakis M, Kouttis A. Release of Furosemide from multiple unit and single unit preparations containing different viscosity grades of sodium alginate. Pharm Dev Tech 2001; 6(1): 91. http://dx.doi.org/ 10.1081/PDT-100000048 PMid:11247279
21. Kumar Ravi, Patil S, Patil MB. Patil SR, Paschapur MS. Isolation and Evaluation of Disintegrant Properties of Fenugreek Seed Mucilage. International Journal of Pharm Tech Research 2009; 1(4): 982-996.

Cite this article as:
Balagani Pavan Kumar, Manubolu Sindhuri, K. Jyothshna Devi, S. Vinoth Kumar, Alluru Manogna, Pichika Madhavi. Isolation and characterization of natural mucilage from Lagenaria siceraria. Int. Res. J. Pharm. 2013; 4(11):117-121 http://dx.doi.org/10.7897/2230-8407.041126

Source of support: Nil, Conflict of interest: None Declared