STM JOURNALS

Research & Reviews: A Journal of Life Sciences
ISSN: 2249-8656 (Online), ISSN: 2348-9545 (Print)
Volume 8, Issue 3
www.stmjournals.com

Bioprospecting Unripe Pericarp of Annona Reticulata for the Presence of Bioactive Compounds

V. S. Sangeetha[1,*], Adheena Elza Johns[2], Beena Lawrence[2]
[1]Department of Biotechnology, Noorul Islam College of Arts and Science, Thuckalay, Kanya Kumari District, Tamil Nadu, India
[2]Department of Botany, Women's Christian College, Nagercoil, Kanya Kumari District, Tamil Nadu, India

Abstract

The present work was carried out with the objective of isolating and identifying biologically significant chemical compounds from the ethanol extract of unripe pericarp of Annona reticulata through bioassay-guided fractionation. Ethanol extract of unripe pericarp was prepared and fractionation was done using silica gel chromatography. Thin-layer chromatography profiling was done and a single compound was obtained. The compound was subjected to various spectroscopic techniques for the elucidation of its structure. The result of spectral analysis of the compound was compared with the reported data which led to propose the structure of bioactive polyphenolic component as ellagic acid. Ellagic acid is reported to have antiproliferative and antioxidant properties. It is also marketed as dietary supplement against cancer, heart disease, and other medical problems.

Keywords: *Bioprospecting, ellagic acid, ethanolic extract, spectroscopic techniques*

****Author for Correspondence*** E-mail: sangeegodwin@gmail.com

INTRODUCTION

Medicinal plants and botanicals have always remained an important resource for the discovery of new drugs. Concept to product journey of such drugs is very challenging and involves various issues starting from bioprospecting to quality, safety, validation, and clinical studies. Bioprospecting has provided important leads to facilitate drug discovery from natural resources [1]. In an effort to expand the spectrum of biologically significant chemical compounds from natural resources, *Annona reticulata* belonging to Annonaceae was selected. *Annona reticulata* has been traditionally used in Indian folk medicines as vermifuge, antiinflammatory agent, and in the treatment of diarrhea and dysentery. Various scientific studies reported its astringent, antianxiety, antistress, and spasmolytic activities [2].

The present work was carried out to isolate and identify biologically significant chemical compounds from the ethanolic extracts of unripe pericarp of *Annona reticulata* through bioassay-guided fractionation.

MATERIALS AND METHODS

Preparation of Ethanol Extract of Unripe Pericarp

Mature unripe fruits of *Annona reticulata* used for the study were collected from Velimalai Hills, Kanyakumari district, Tamil Nadu, during the month of June to September. The pericarp of the fruits were separated from the seeds, cut into small pieces, shade dried, and powdered. The powder was soaked in ethanol for 48 hours and filtered [3].

Fractionation and Thin-Layer Chromatography

Fractionation was done using silica gel column chromatography [4]. Fractions were then collected, concentrated, and those having similar R_f value on the thin-layer chromatography (TLC) plates were pooled, numbered, and TLC profiling was done to get a single compound.

Spectroscopic Studies of Active Fraction

The isolated compound was subjected to various spectroscopic techniques for elucidation of the structure; namely, UV-visible spectral analysis, Infrared spectral analysis, ^1H

and ^{13}C-Nuclear Magnetic Resonance (NMR) spectrum analysis and electrospray ionization mass spectrometry (ESI-MS) analysis.

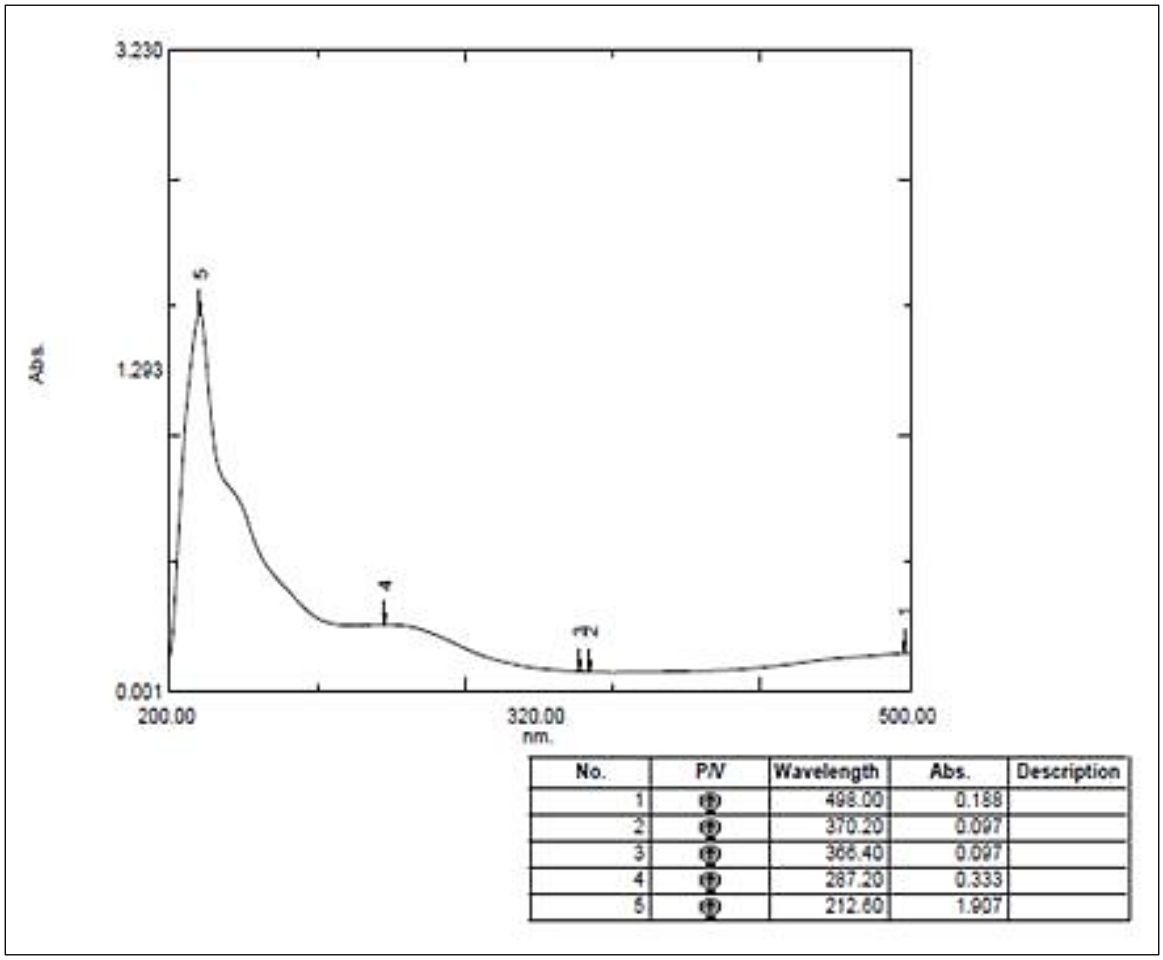
RESULTS AND DISCUSSION

TLC of a single compound isolated from ethanol extract of unripe pericarp of *Annona reticulata* under UV light was conducted and a single spot was obtained with the fraction, the R_f value of which was determined as 0.64. The isolated compound was subjected to various spectroscopic techniques for the elucidation of the structure.

Spectral Analysis of Active Fraction of Ethanol Extract

Figures 1 to 5 presents the results of spectral analysis carried out on the active fraction of ethanol extract. The UV spectrum of the active fraction showed absorption maxima at 253 nm and 366 nm. The infrared spectrum of the compound exhibited broad band in the range, 2924-3556 cm^{-1} which is attributed to the –OH stretching, while the band observed at 1697cm^{-1} corresponds to C=O stretching. The bands observed in the range, 1614-1510cm^{-1} are due to aromatic ring vibrations (C=C), while the ones at 1195 cm^{-1} and 1055 cm^{-1} are due to ester linkages (C-O). The band at 757 cm^{-1} is assigned to aromatic C-H bending vibration. The ESI-MS spectrum showed a single strong peak at (*m/z*) 301$[M+H]^+$ g/mol corresponding to ellagic acid.

^{1}H NMR (DMSO, 500 MHz): ^{1}H NMR spectrum of the active fraction showed a peak at 10.5 and 10.7 ppm which corresponds to –OH proton (4H, s). The peak at 7.46 ppm corresponds to aromatic C–H protons (2H). The peaks at 3.4 and 2.5 ppm correspond to a solvent proton, respectively. ^{13}C NMR (DMSO, 126 MHz) spectroscopy showed δ value at 159.14 (C-7, 7'), 148.11 (C-4, 4'), 139.55 (C-3, 3'), 136.39 (C-2, 2'), 112.31(C-1, 1'), 110.27 (C-5, 5') and 107.69 (C-6, 6'). The peaks from 38 to 40 ppm correspond to solvent peaks.



No.	P/V	Wavelength	Abs.	Description
1		498.00	0.188	
2		370.20	0.097	
3		366.40	0.097	
4		287.20	0.333	
5		212.60	1.907	

Fig. 1: *UV Spectrum.*

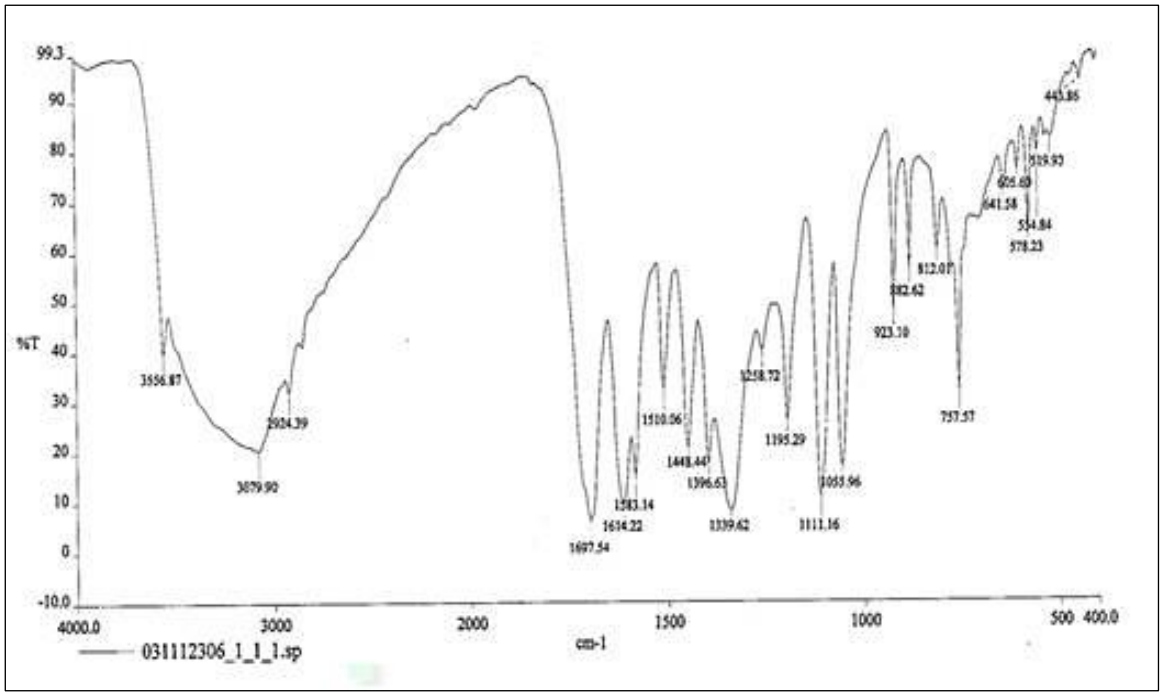


Fig. 2: *Infrared Spectrum.*

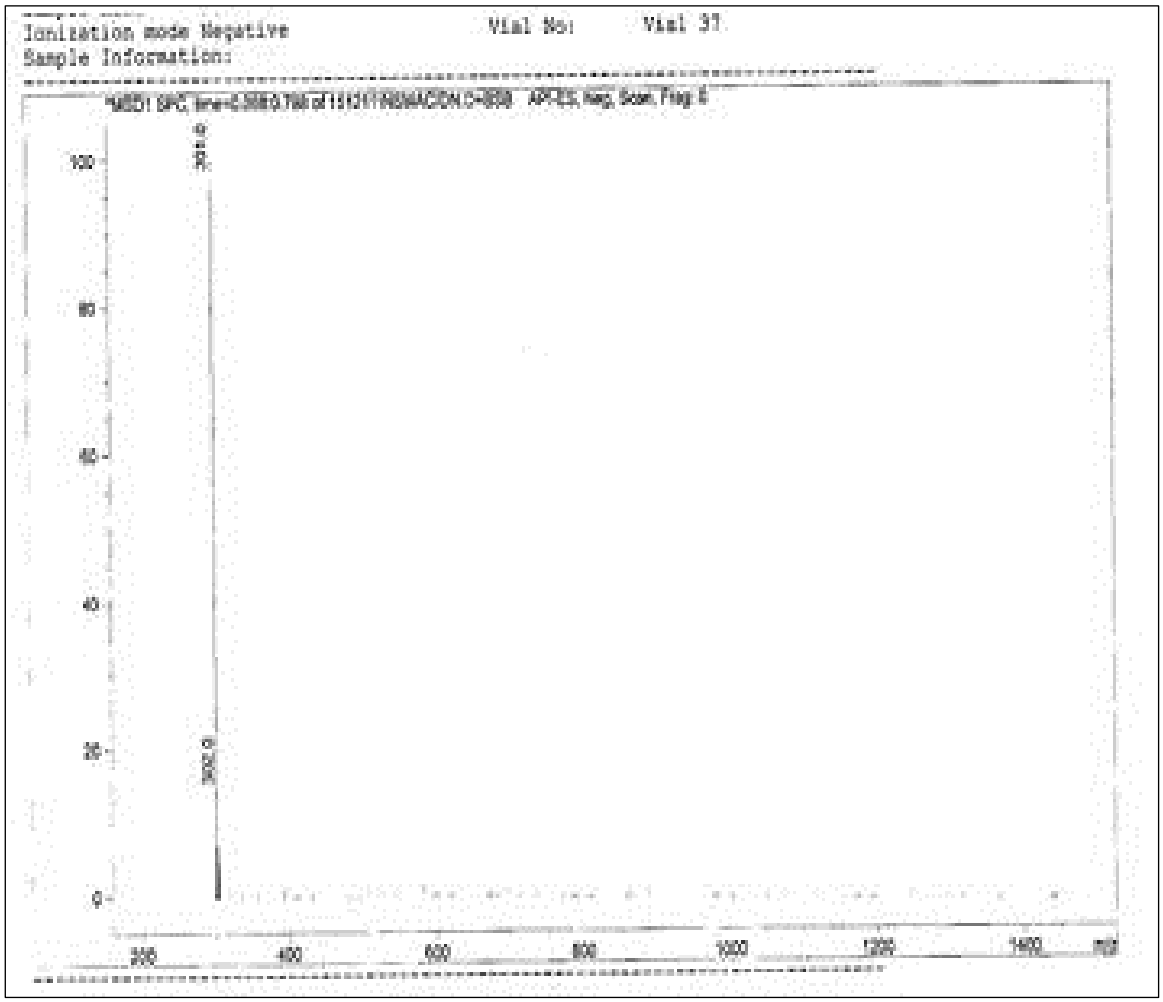


Fig. 3: *ESI-MS Spectrum.*

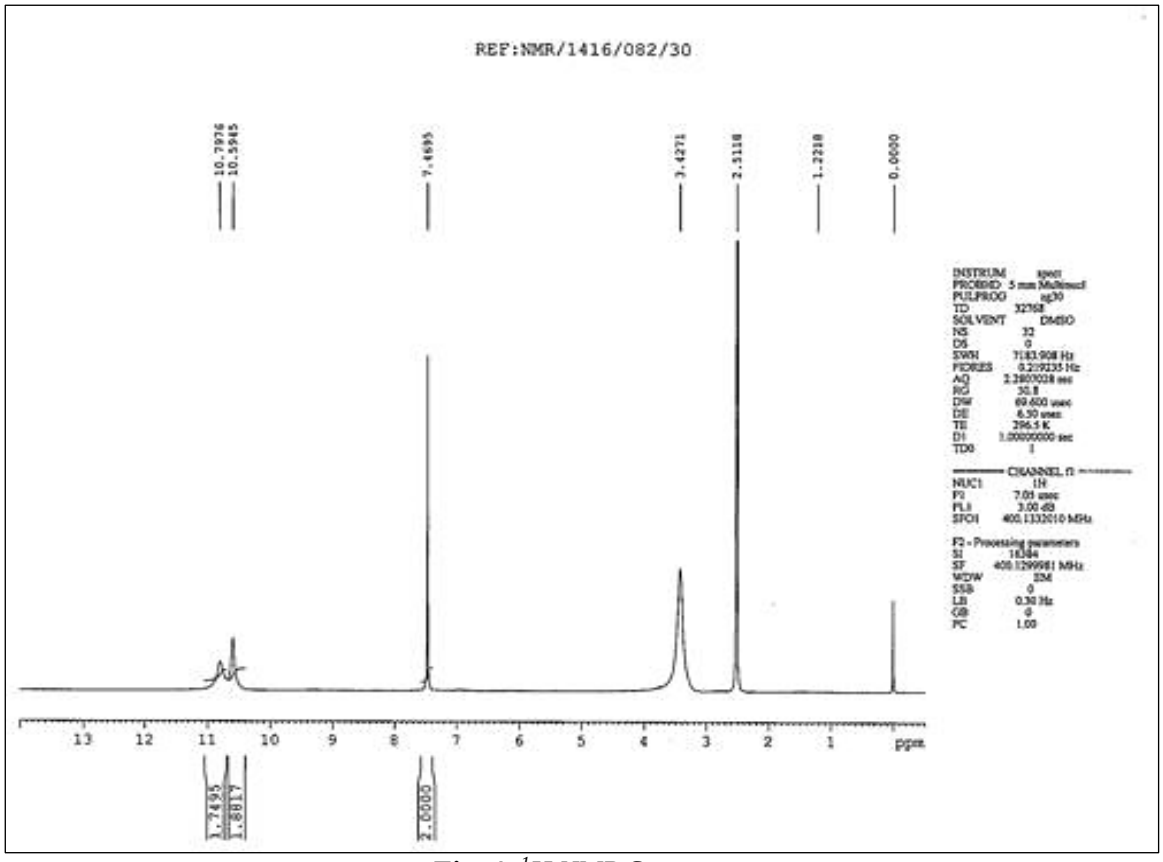


Fig. 4: *^{1}H NMR Spetrum.*

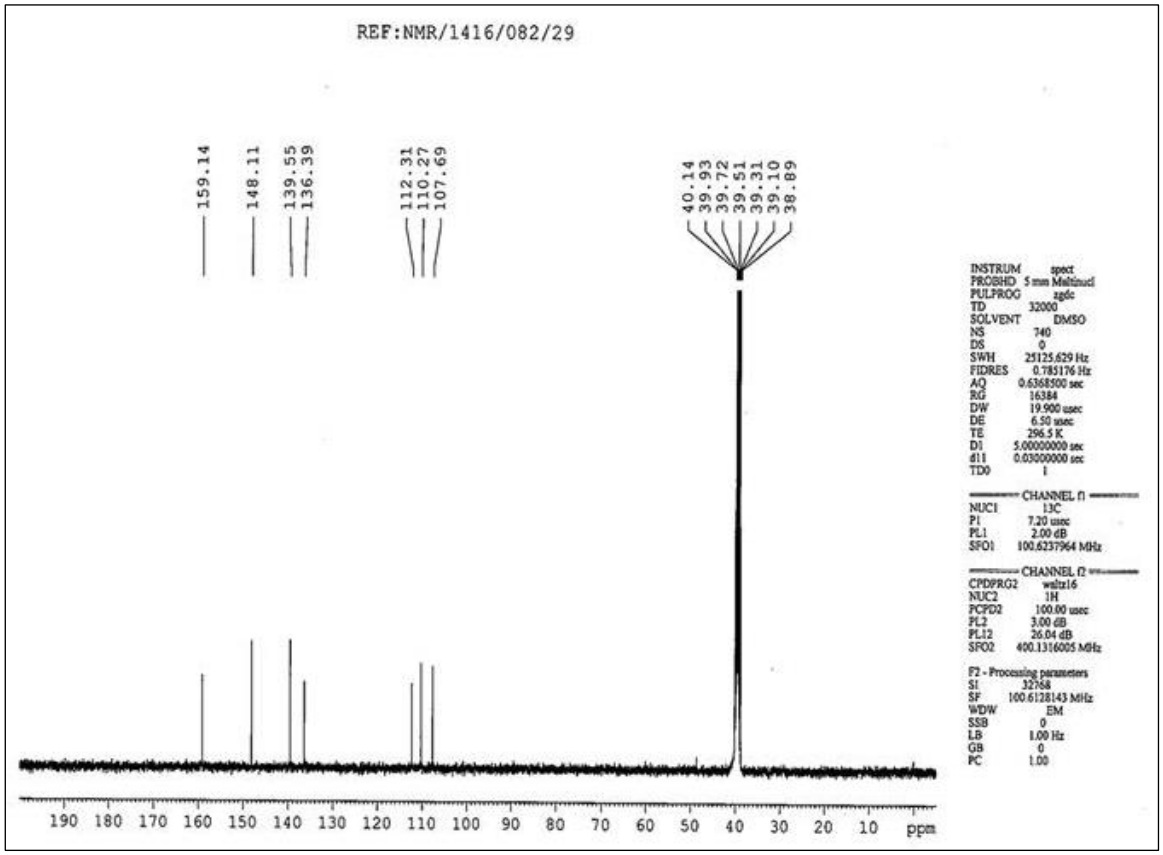


Fig. 5: *^{13}C NMR Spectrum.*

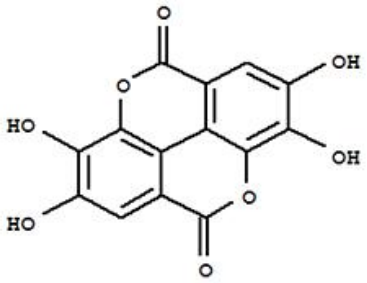


Fig. 6: *Ellagic Acid.*

The spectral analysis and the further comparison with the reported data led us to propose the structure of bioactive compound as ellagic acid (Molecular formula: $C_{14}H_6O_8$), which is a polyphenolic compound (Figure 6).

Bioassay-guided fractionation has played an important role in the isolation of active compounds/fractions from natural products [5,6]. In the present study, bioassay-guided fractionation was employed to isolate the bioactive fractions/compounds from the unripe pericarp. The isolated fraction was subjected to ultraviolet, infrared, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. Two aromatic ring proton(s) singlets d 7.45 ppm (2H, s, 4, 9- H) and four broad hydroxyl signals d 10.6 (2H, br) and 4.10 ppm (2H, br), which disappeared on deuterium exchange, were observed in its ^{1}H NMR spectra. In its ^{13}C NMR spectra, a set of seven carbon signals were observed. Two ppm indicated a carbonyl group, while the 148.1 to 107.7 ppm suggested the presence of a benzene ring including those for the 5-carbon units of the gallic acid skeleton. Thus, a symmetrical molecule was identified on the basis of its mass spectrum and NMR data. It was further confirmed as ellagic acid by comparison with the literature [7]. Thus, the results of spectral data suggest that the bioactive lead obtained was ellagic acid. The presence of ellagic acid has been reported in several medicinal plants [8,9,10,11]. Its presence in human dietary sources, especially fruits and nuts also has been reported [12,13,14].

Ellagic acid has been reported to have antiproliferative and antioxidant properties in a number of *in vitro* and small animal models and also prevents the destruction of p53 gene by cancer cells [15,16]. There are also reports on its chemoprotective effect in cellular models by reducing oxidative stress [17].

CONCLUSIONS

The polyphenolic compound isolated from unripe pericarp of *Annona reticulata* identified as ellagic acid has been reported to be having anticancerous and antioxidant properties and hence the unripe pericarp can be used as a biological resource for producing drugs. The anticancerous activity and cytotoxicity of the ethanolic unripe pericarp extracts of *Annona reticulata* has to be screened using cancer cell lines. This might contribute to the development of valuable anticancer drug. Furthermore, the fractionated compound flashes more light into the pharmacological efficiency of the plant studied.

CONFLICT OF INTEREST

Nil

AUTHORS'S CONTRIBUTIONS

Mrs. V. S. Sangeetha carried out the study design and bioassay-guided fractionation of the extract and drafted the manuscript. Ms. Adheena Elza Johns helped in analyzing the spectral characteristics of the isolated compound. Dr. Beena Lawrence, the research guide participated in coordinating the drafted manuscript and helped to draft the final manuscript. All authors read and approved the final manuscript.

REFERENCES

1. Ankur P, Makarand P, Radhika J. Evaluation of anti-diabetic property of extracts of different plant parts of Salacia chinensis Linn. *J Biodivers Biopros Dev*. 2014; 1 (1): 1–4p.
2. Kirtikar KR, Basu, BD. *Indian medicinal plants*. Allahabad, India: Bishan Singh Mahendra Pal Singh; 1991.
3. Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. *J Pharmacogn Phytochem*. 2014; 2 (5): 115–119p.
4. Harbourne JB. Phytochemical methods: A guide to modern techniques of plant analysis, 2nd ed. London, England: Chapman and Hall Publications; 1984.
5. Ikan R. *Natural products*, 2nd ed., New York, NY: Academic press; 1991.
6. Spiegler V, Sendker J, Petereit F, Liebau E, Hensel A. Bioassay-guided fractionation of

a leaf extract from *Combretum mucronatum* with anthelmintic activity: Oligomeric Procyanidins as the active principle. *Molecules*. 2015; 20: 14810–14832p.
7. Lan HQ. Study on the chemical constituents from *Sarcopyramis nepalensis*. *Zhong Yao Cai*. 2010; 33 (4): 547–549p.
8. Aleksandra O, Monika AO, Gudej J. Quantitative determination of ellagic acid and gallic acid in *Geum rivale* and *Geum urbanum*. *Acta Biol Cracov Ser Bot*. 2014; 56 (2): 74–78p.
9. Ann EH. Quantitative determination of ellagic acid. *J. Agric Food Chem*. 1990; 38 (8): 1678–1683p.
10. Dhooghe L, Meert H, Cimanga RK, Vlietnick AJ, Pieters L, APers S. The quantification of ellagic acid in the crude extract of *Phyllanthus amarus* Schum. & Thonn. (Euphorbiaceae). *Phytochem Anal*. 2011; 4: 361–366p.
11. Soong YY, Barlow PJ. Quantification of gallic acid and ellagic acid from longan (*Dimocarpus longan* Lour.) seed and mango (*Mangifera indica* L.) kernel and their effects on antioxidant activity. *Food Chem*. 2006; 97: 524–530p.
12. Wang SY, Maas JL, Daniel, EM, Galetta, GJ. Improved HPLC resolution and quantification of ellagic acid from strawberry, blackberry and cranberry. *Horti Sci*. 1990; 25: 1078p.
13. Boyle JA, Hsu L. Identification and quantification of ellagic acid in muscadine grape juice. *Am J Enology Vitic*. 1990; 4: 43–47p.
14. Daniel EM, Krupnick AS, Heur YH, Blinzler JA, Nims RW, Stoner, GD. Extraction, stability and quantitation of ellagic acid in various fruits and nuts. *J Food Compos Anal*. 1989; 2, 338–349p.
15. Seeram NP, Adams LS, Henning SM. *In vitro* anti proliferative, apoptotic and antioxidant activities of punicalegin, ellagic acid and a total pomegranate tannin extract enhanced in combination with other polyphenols as found in pomegranate ice. *J Nutri Biochem*. 2005; 16 (6): 360–370p.
16. Oszmiański J, Nowicka P, Teleszko M, Wojdyło A, Cebulak T, Oklejewicz K. Analysis of phenolic compounds and antioxidant activity in wild blackberry fruits. *Int J Mol Sci*. 2015; 16 (7): 14540–14553p.
17. Vattem DA, Shetty K. Biological function of ellagic acid: A review. *J Food Biochem*. 2005; 29 (3): 234–266p.

Cite this Article
V. S. Sangeetha, Adheena Elza Johns, Beena Lawrence. Bioprospecting Unripe Pericarp of Annona Reticulata for the Presence of Bioactive Compounds. *Research & Reviews: A Journal of Life Sciences*. 2018; 8(3): 44–49p.