IJPSR (2021), Volume 12, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 12 October 2020; received in revised form, 22 February 2021; accepted, 24 May 2021; published 01 September 2021

ANTI-INFLAMMATORY ACTIVITY POTENTIAL OF *ARECA CATECHU* LEAF EXTRACTS- AN *IN-VITRO* STUDY

N. G. D' Souza [1], J. Fernandes [*2] and S. D' Souza [1]

Department of Pharmaceutical Chemistry [1], Yenepoya Pharmacy College and Research Centre, Yenepoya (Deemed to be University), Deralakatte, Mangalore - 575018, Karnataka, India.
Department of Pharmaceutical Chemistry [2], NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Paneer, Deralakatte, Mangalore - 575018, Karnataka, India.

Keywords:

Areca catechu, Antioxidant, Anti-inflammatory activity

Correspondence to Author:
Dr. Jennifer Fernandes

Department of Pharmaceutical Chemistry, Yenepoya Pharmacy College and Research Centre, Yenepoya (Deemed to be University), Deralakatte, Mangalore - 575018, Karnataka, India.

E-mail: fernandesj@nitte.edu.in

ABSTRACT: *Areca catechu* L. (Arecaceae) is a species of palm mainly grown in Asian countries for seed crop. Areca nut leaves are commonly used in traditional systems of medicine against various ailments. There is a need for identifying native natural plant sources to acquire their recognized medicinal properties, which may widen them to use as new therapeutics for various diseases. Phytochemical investigation of the leaves of *Areca catechu* L. yielded a alkaloid- Arecoline, from the chloroform extract; a flavonoid- Apigenin, from the methanol extract and a steroid- Stigmasterol, from petroleum ether extract. *In-vitro* methods were used to evaluate the pharmacological activity of the leaves of *Areca catechu* L. Anti-inflammatory activity of the leaves of Areca catechu was estimated by using standard methods like protein denaturation method using bovine serum albumin fraction and egg albumin and by HRBC membrane stabilization method. The result suggests that the leaves possess good antioxidant as well as anti-inflammatory activity.

INTRODUCTION: Herbal medicine, also known as phytotherapy, is the practice of treating different illnesses with natural remedies. This involves all facets of herbal medicine, *i.e.* plants with strong acts for people with gentle actions [1]. Herbal medicines is the key of traditional medicine systems like Ayurveda, Sidha and Unani [2]. Traditional medicine, especially herbal medicine, is seen as a major provider of healthcare around the world, particularly in rural and remote areas [3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(9).5065-71

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(9).5065-71

For the treatment of different health problems, medicinal plants appear to be an interesting source of natural products [4, 5]. Even with the advances of the modern medicines, in many developing countries herbal medicines play a major role in the treatment of many diseases. In India around 85% of the population still uses the crude plant preparations for treating various types of ailments and diseases. The plant material which is used as the API in the herbal formulations is readily available in and around the rural areas which has made herbal medicines cheaper compared to the modern medicines [6].

Inflammation is the process of defence triggered by living tissue during an illness or injury. This initiates phase of healing by removing the injurious agents and around tissue debris [7].

Anti-inflammatory drugs are classified as agents that either prevent or decrease inflammation that can occur as a result of interactions between physical damage, infection, heat and antigen-antibody [8].

Steroidal and non-steroidal anti-inflammatory medications that, when used as long-term therapies, may cause significant side effects, such as gastrointestinal and peptic ulcer bleeding [9]. An alternative source of new compounds with anti-inflammatory activity is natural products [10]. Medicinal plants play an important role in the development of new and potent drugs due to the availability of secondary metabolites with clinical curative effects [11]. *Areca catechu* L. (Family: Arecaceae) commonly known as Areca palm or Betel nut palm or Betel palm. These are widely cultivated and grown more or less throughout the year in India and many other Asian countries [12]. The name betel nut palm or betel palm is because, its fruits are often chewed with the betel leaf and lime [13]. The fruits of the palm are traditionally used in diarrhoea, urinary disorders, digestive aid, expelling intestinal worms, stimulant effect and breathe freshening effect [14]. While the nuts of the tree have been reported for many activities there is no literature available for the isolation of phytoconstituents of the leaves of the plant. To confirm the active constituents of the leaves, the present study is designed. With this background the leaves of the plant are selected for screening of anti-inflammatory activity [15].

MATERIALS AND METHODS:

Plant Collection: The leaves of Areca catechu required for the study was collected from in and around Mangalore, Dakshina Kannada (dist), Karnataka; in the month of June and July 2017. The leaves were authenticated by Dr. K. V. Nagalakshamma, Head of the Dept. of Botany, St. Aloysius College, Mangalore. A voucher specimen (No.16PC005) is deposited in NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore.

Extraction: The leaves of Areca catechu L. were cleaned and shade dried for about 7 days. The shade dried leaves were then ground into coarse powder using a mechanical grinder. The coarse powder of leaves of *Areca catechu* L. were subjected to cold maceration extraction using chloroform and methanol as the solvents [16]. Cold maceration was done in two parts of the plant powder (100 g) for all the solvents, each for 7 days. After the extraction, the macerated powder was filtered using a muslin cloth. The marc obtained was air-dried. The filtrate obtained was subjected to steam distillation, to concentrate the extract and the solvent was recollected and was used for further extraction process. A dark green residue was obtained on further concentrating and evaporating the extract on a water bath. The dried extract thus obtained was kept in the desiccator and was used for further phytochemical and pharmacological investigation.

Preliminary Phytochemical screening Qualitative Analysis: The chloroform, methanol extracts of leaves of *Areca catechu* L. was subjected to preliminary qualitative phytochemical screening, to determine the different chemical groups present in it. Chemical tests were carried out as per the standard methods [17].

Screening of Anti-Inflammatory Activity By *In-vitro* Methods: Denaturation of tissue proteins and lysis of RBC membrane are the prominent causes of inflammatory diseases. During inflammation, there are lyses of lysosomes which release their component enzymes that produce a variety of disorders. Non-steroidal anti-inflammatory drugs (NSAIDs) exert their beneficial effects by either inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membranes [18].

These drugs in spite of their potency in relieving pain are also associated with some serious side effects on prolonged duration of usage which include gastric bleeding, bone marrow disturbance, ulceration, liver and kidney dysfunction [19]. However, plant-derived drugs are used to treat most of the inflammatory diseases even today because of their least or no side effects [20].

Protein Denaturation by Bovine Serum Albumin Method: The reaction mixture consisting of test extracts and 1% aqueous solution of bovine albumin fraction was prepared. The pH of the reaction mixture was adjusted using a small amount of 0.1N HCl. Sample mixture consists of 0.45 ml of bovine serum albumin solution and 0.05 ml of the extract solution [21].

0.5 ml of this sample mixture was taken in test tubes and were incubated at 37 °C for 20 min and then to the above sample mixture 2.5 ml of phosphate buffer saline was added.

The absorbance of the sample mixture was taken at 660 nm. Percentage inhibition of protein denaturation was calculated using the following formula.

$$\% \text{ inhibition} = (Abs_{control} - Abs_{test} / Abs_{control}l) \times 100$$

Where $Abs_{control}$ is the absorbance of the control and Abs_{test} is the absorbance in the presence of test extracts

Protein Denaturation by Egg Albumin Method: The reaction mixture consisting of 0.45 ml of 5% egg albumin solution and 0.05 ml of the test extract solution was taken in a test tube and these tubes were incubated at 37 °C for 20 min.

After incubation to the above solution 2.5 ml of the phosphate buffer saline was added. The absorbance of the reaction mixture was taken at 660 nm. Percentage inhibition of protein denaturation was calculated using the following formula [21].

$$\% \text{ inhibition} = (Abs_{control} - Abs_{test} / Abs_{control}l) \times 100$$

Where $Abs_{control}$ is the absorbance of the control and Abs_{test} is the absorbance in the presence of test extracts.

Human Red Blood Cell Membrane Stabilization Method:

Collection of blood sample: The human red blood cells required for the experiment was collected from a healthy volunteer who had not consumed any anti-inflammatory drugs (NSAID's) for 2 weeks prior to the experiment [22, 23].

***In-vitro* Anti-Inflammatory Activity:**

Bovine Serum Albumin Denaturation Method:

Preparation of Human Red Blood Cell (HRBCS) Suspension: The fresh whole human blood (10 ml) collected was transferred to the centrifuge tubes with equi volume of Alsever's solution. The tubes were centrifuged at 3000 rpm for 10 min and the packed cells were washed thrice with equal volume of iso-saline solution. The volume of the cells was measured and a 10% v/v suspension was reconstituted using iso-saline solution [24].

Heat-Induced Haemolysis: 1 ml of the test extract each of concentration ranging from 50-300 µg/ml, was taken in a test tube, to this 1 ml of freshly prepared phosphate buffer was added along with 2 ml of saline solution. Finally, 0.5 ml of freshly prepared HRBC suspension was added. Diclofenac sodium was used as the standard drug and in the control tube saline was taken omitting the test sample. All the test tubes were incubated at 37 °C for 30 min. After incubation, the contents were then centrifuged at 3000 rpm for 20 min. The supernatant solution was collected and was estimated spectrophotometrically at 560 nm. The percentage inhibition of lysis was calculated using the following formula.

$$\% \text{ inhibition} = (Abs_{control} - Abs_{test} / Abs_{control}) \times 100$$

Where $Abs_{control}$ is the absorbance of the control and Abs_{test} is the absorbance in the presence of test extracts

Results:

Preliminary Phytochemical Investigation: Preliminary phytochemical screening of both the chloroform extract and methanol extract of the leaves of Areca catechu revealed the presence of alkaloids, carbohydrates, flavonoids, triterpenoids, resins, steroids and tannins.

TABLE 1: EFFECT OF CHLOROFORM AND METHANOLIC EXTRACT OF ARECA CATECHU ON BOVINE SERUM ALBUMIN DENATURATION

Tested material	Concentration (µg/ml)	% Inhibition on BSA denaturation ± SEM	IC_{50} value
Diclofenac	50	16.75±0.020	
	100	34.51±0.012	
	150	50.76±0.013	110.19
	200	62.94±0.011	
	250	74.61±0.016	

	300	84.26±.0100	
Chloroform extract of *Areca catechu*	50	11.67±0.011	
	100	22.84±0.013	
	150	46.70±0.019	131.95
	200	58.37±0.012	
	250	69.54±0.025	
	300	79.18±0.017	
Methanolic extract of *Areca catechu*	50	13.19±0.019	
	100	24.36±0.014	
	150	48.73±0.022	125.60
	200	59.89±0.020	
	250	72.08±0.016	
	300	80.71±0.013	

All values are expressed in terms of ± SEM and are found to be significant when compared to control P<0.05

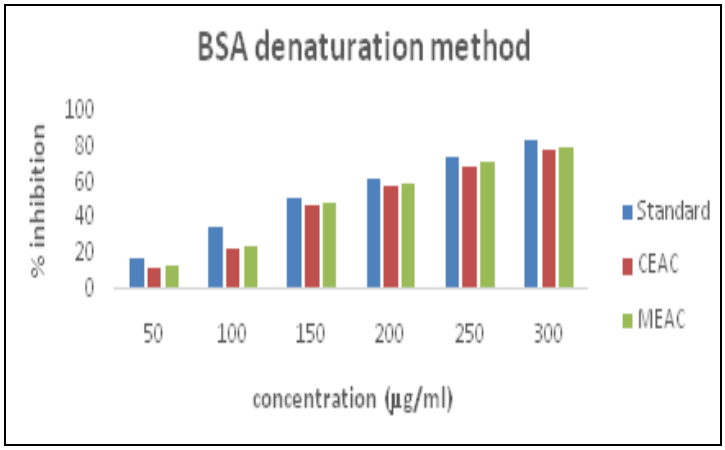

FIG. 1: BSA DENATURATION METHOD

Egg Albumin Denaturation Method:

TABLE 2: EFFECT OF THE CHLOROFORM AND METHANOLIC EXTRACT OF ARECA CATECHU ON EGG ALBUMIN DENATURATION

Tested material	Concentration (µg/ml)	% Inhibition on Egg albumin denaturation ± SEM	IC_{50} value
Diclofenac	50	13.47±0.010	
	100	25.38±0.005	
	150	38.86±0.012	137.61
	200	56.99±0.009	
	250	66.32±0.012	
	300	78.75±0.011	
Chloroform extract of *Areca catechu*	50	8.80±0.008	
	100	19.68±0.015	
	150	34.19±0.014	159.06
	200	49.74±0.011	
	250	63.21±0.015	
	300	70.98±0.010	
Methanolic extract of *Areca catechu*	50	11.39±0.018	
	100	23.31±0.015	
	150	37.30±0.010	146.36
	200	51.81±0.011	
	250	65.28±0.010	
	300	76.68±0.015	

All values are expressed in terms of ± SEM and are found to be significant when compared to control P<0.05

HRBC Membrane Stabilization Method:

TABLE 3: EFFECT OF CHLOROFORM AND METHANOLIC EXTRACT OF ARECA CATECHU ON HRBC MEMBRANE STABILIZATION TEST

Tested material	Concentration (µg/ml)	% Inhibition on membrane stabilization ± SEM	IC_{50} value
Diclofenac	50	15.09±0.021	
	100	26.41±0.027	
	150	37.73±0.026	152.64
	200	50.00±0.017	
	250	62.26±0.020	
	300	70.75±0.022	
Chloroform extract of *Areca catechu*	50	12.26±0.022	
	100	23.58±0.024	
	150	26.41±0.024	185.97
	200	38.67±0.028	
	250	54.38±0.025	
	300	66.03±0.021	
Methanolic extract of *Areca catechu*	50	13.20±0.026	
	100	24.52±0.019	
	150	32.07±0.027	167.87
	200	46.22±0.020	
	250	57.54±0.028	
	300	68.86±0.023	

All values are expressed in terms of ± SEM and are found to be significant when compared to the control P<0.05.

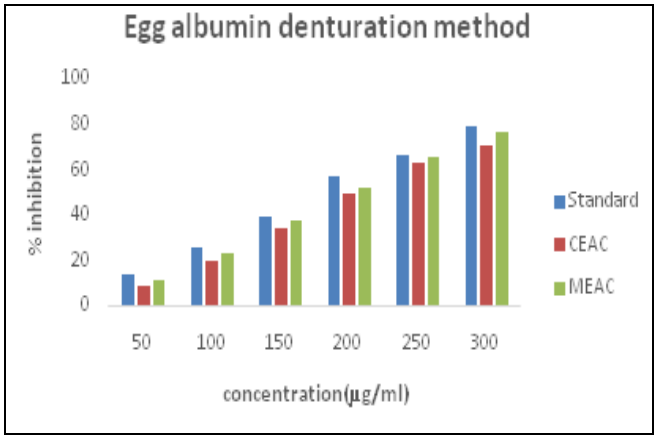

FIG. 2: EGG ALBUMIN DENATURATION METHOD

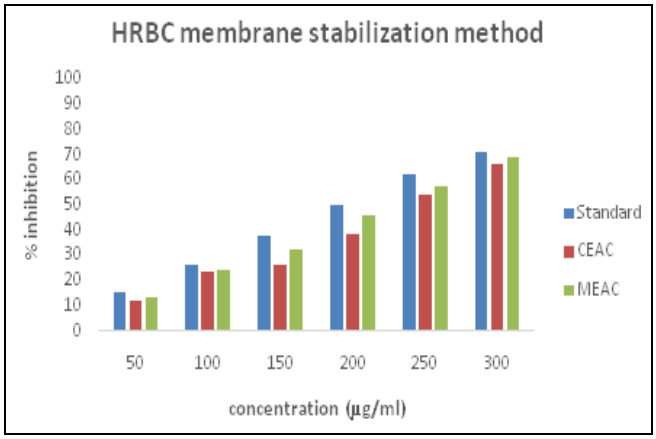

FIG. 3: HRBC MEMBRANE STABILIZATION METHOD

Discussion:

***In-vitro* Anti-Inflammatory Studies**

Bovine Serum Albumin Denaturation Assay: The inhibition of denaturation of bovine serum albumin by heat was studied with chloroform as well as methanolic extract of the leaves of Areca catechu as the test sample and diclofenac sodium as the standard drug.

The extracts showed concentration dependant increase in the percentage inhibition values as similar to that of the standard drug. The IC_{50} value of chloroform extract was found to be 131.95 and that of methanolic extract was 125.60, whereas the standard value was 110.19. The absorbance values decreased as the concentration increased, which was recorded at 660 nm, showed significant anti-inflammatory activity.

Egg Albumin Denaturation Assay: In this method, the only difference when compared to bovine serum albumin denaturation assay is that the bovine serum albumin fraction was replaced with egg albumin. The percentage inhibition of leaves extract of Areca catechu increased with increasing concentration. The IC_{50} values of chloroform extract were found to be 159.06, and that of methanolic extract was found to be 146.36, whereas the standard value was 137.61. The decrease in the absorbance value with the increasing concentration taken at 660 nm shows significant anti-inflammatory activity.

HRBC Membrane Stabilization Method: The chloroform and methanolic extract of leaves of Areca catechu showed concentration dependant increase in percentage inhibition with HRBC membrane stabilization method. The IC_{50} values of chloroform extract were found to be 185.97, and that of methanolic extract was found to be 167.87, whereas the standard value was 152.64. The decrease in the absorbance value with an increase in concentration suggests that the anti-inflammatory activity is observed in this study.

CONCLUSION: Preliminary phytochemical screening of chloroform and methanolic extracts of leaves of Areca catechu gave positive results for the presence of alkaloids, reducing sugars, flavonoids, triterpenoids, glycosides, resins, steroids, and tannins; and gave negative results for saponins and proteins. Anti-inflammatory activity of the leaves of Areca catechu was estimated by using standard methods like protein denaturation method using bovine serum albumin fraction and egg albumin; and by HRBC membrane stabilization method.

The chloroform extract, as well as the methanolic extract of the Areca catechu, showed significant percentage inhibition when compared to the control. Diclofenac sodium was used as the standard drug. The *in-vitro* anti-inflammatory activity showed positive results, thus promoting their use in the management of inflammation. From the studies carried out on leaves of *Areca catechu* L., it is evident that it has significant anti-inflammatory activity. The side effects caused by the currently available drugs like NSAIDs and opioid analgesics used in managing pain and inflammation made to search for newer agents with less toxicity, more effective, and more economical, thus promoting their use in the traditional system of medicine.

The results obtained in the present study are encouraging and offer the scope of further study. In any case where a group of plant part which are reported to be used in the treatment of any specific disease contain relatively higher levels of a specific element in comparison with other samples. These propositions offer further studies on these plants, constituents concerned and the corresponding diseases. Suitable animal experiments could be designed to further investigate the efficacy of these plants in treating the diseases and also the remedial roles of these elements.

ACKNOWLEDGEMENT: The authors are grateful to NGSM Institute of Pharmaceutical Sciences and Nitte (Deemed to be University) for providing the necessary facilities to carry out the research work.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

REFERENCES:

1. D' Souza S, Fernandes J, Kumar MV, D'Souza NG and Fernandes R: Antiepileptic activity of ethanolic and aqeous leaves extract of *Annona reticulata* Linn. Research Journal of Pharmacy and Technology 2019; 12(1): 241-4.
2. Bhatt N: Ayurvedic drug industry (challenges of today and tomorrow. Proceeding of the first national symposium of ayurvedic drug industry organised by (ADMA). Ayurvedic New Delhi sponsored by Department of Indian Systems of Medicine oh HOM Ministry of Health Govt of India Aug New Delhi 1999.
3. Sen S and Chakraborty R: Revival, modernization and integration of Indian traditional herbal medicine in clinical practice: Importance, challenges and future. Journal of Traditional and Comple Medicine 2017; 1: 7(2): 234-44.
4. Nunes CD, Barreto Arantes M, Menezes de Faria Pereira S, Leandro da Cruz L, de Souza Passos M, Pereira de Moraes L, Vieira IJ and Barros de Oliveira D: Plants as Sources of Anti-Inflammatory Agents Molecules 2020; 25(16): 3726.
5. Shazhni JA, Renu A and Vijayaraghavan P: Insights of antidiabetic anti-inflammatory and hepatoprotective properties of antimicrobial secondary metabolites of corm extract from Caladium x hortulanum. Saudi Journal of Biological Sciences 2018; 25(8): 1755-61.
6. Amudhan MS, Begum VH and Hebbar KB: A review on phytochemical and pharmacological potential of *Areca catechu* L seed. International Journal of Pharmaceutical Sciences and Research 2012; 3(11): 4151.
7. Kodical DD, Fernandes J and Deepthi K: *In-vitro* Anti-Inflammatory activity of mirabilis Jalapa flower extracts. Plant Archives 2020; 20(2): 8997-00.
8. Sahlan M, Devina A, Pratami DK, Situmorang H, Farida S, Munim A, Kusumoputro B, Yohda M, Faried A, Gozan M and Ledyawati M: Anti-inflammatory activity of tetragronula species from Indonesia. Saudi Journal of Biological Sciences 2019; 26(7): 1531-8.
9. Amri O, Zekhnini A, Bouhaimi A, Tahrouch S and Hatimi A: Anti-inflammatory activity of methanolic extract from *Pistacia atlantica* desf. leaves. Pharmacognosy Journal 2018; 10(1): 71-76.
10. Villagómez-Rodríguez A, Pérez-Ramos J, Esquivel-Campos AL, Pérez-González C, Soto-Peredo CA and Pérez-Gutiérrez S: Anti-inflammatory activity of *Jefea gnaphalioides* (a. gray), Astereaceae. BMC complementary and alternative medicine. 2019; 19(1): 1-8.
11. Li Y, Kong D, Fu Y, Sussman MR and Wu H: The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiology and Biochemistry 2020; 148: 80-9.

12. Dsouza NG, Fernandes J, D'Souza S and Fernandes R: *In-vitro* antioxidant activity of leaves extracts of areca catechu. Research Journal of Pharmacy and Technology 2019; 12(4): 1536-40.
13. Zhou B, Zhu W and Ren C: First steps to regulate advertising of Areca nut in China. The Lancet Oncology 2019; 20(5): 615-16.
14. Salehi B, Konovalov DA, Fru P, Kapewangolo P, Peron G, Ksenija MS, Cardoso SM, Pereira OR, Nigam M, Nicola S and Pignata G: Areca catechu-from farm to food and biomedical applications. Phytotherapy Research 2020; 34(9): 2140-58.
15. Jaiswal P, Kumar P, Singh VK and Singh DK: Areca catechu L: A valuable herbal medicine against different health problems. Research Journal of Medicinal Plants 2011; 5(2): 145-52.
16. Madhumita M, Guha P and Nag A: Bio-actives of betel leaf *Piper betle* L: A comprehensive review on extraction isolation, characterization, and biological activity. Phytotherapy Research 2020; 34(10): 2609-27.
17. Joseph BS, Kumbhare PH and Kale MC: Preliminary phytochemical screening of selected medicinal plants. Int Res J Sci Eng 2013; 1(2): 55-62.
18. Vallecillo-Hernández J, Barrachina MD, Ortiz-Masiá D, Coll S, Esplugues JV, Calatayud S and Hernández C: Indomethacin disrupts autophagic flux by inducing lysosomal dysfunction in gastric cancer cells and increases their sensitivity to cytotoxic drugs. Scientific Reports 2018; 26; 8(1): 1-10.
19. Rogoveanu OC, Streba CT, Vere CC, Petrescu L and Trăistaru R: Superior digestive tract side effects after prolonged treatment with NSAIDs in patients with osteoarthritis. Journal of Medicine and life 2015; 8(4): 458.
20. Sheetal V and Singh SP: Current and future status of herbal medicines. Veterniary World 2008; 1(11): 347-50.
21. Djuichou Nguemnang SF, Tsafack EG, Mbiantcha M, Gilbert A, Atsamo AD, Yousseu Nana W, Matah Marthe Mba V and Adjouzem CF: *In-vitro* anti-inflammatory and *in-vivo* antiarthritic activities of aqueous and ethanolic extracts of *Dissotis thollonii* Cogn.(Melastomataceae) in rats. Evidence-Based Complementary and Alternative Medicine. 2019; 15; 2019.
22. Govindappa M, Bharath N, Shruthi HB, Sadananda TS, Sharanappa P. Antimicrobial, antioxidant and *in-vitro* anti-inflammatory activity and phytochemical screening of Crotalaria pallid Aiton. Afr J Pharm Pharmacol 2011; 5(21): 2359-71.
23. Yesmin S, Paul A, Naz T, Rahman AA, Akhter SF, Wahed MI, Emran TB and Siddiqui SA: Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (*Piper chaba*). Clinical Phytoscience. 2020; 6(1): 1-10.
24. Leelaprakash G and Dass SM: *In-vitro* anti-inflammatory activity of methanol extract of *Enicostemma axillare*. Int J of Drug Development and Research. 2011; 3(3): 189-96.

How to cite this article:
D' Souza NG, Fernandes J and D' Souza S: Anti-inflammatory activity potential of *Areca catechu* leaf extracts-an *in-vitro* study. Int J Pharm Sci & Res 2021; 12(9): 5065-71. doi: 10.13040/IJPSR.0975-8232.12(9).5065-71.