World Inventia Publishers

Journal of Pharma Research

http://www.jprinfo.com/

Vol. 7, Issue 5, 2018 ISSN: 2319-5622 USA CODEN: JPROK3

Research Article

STUDIES ON PHYTOCHEMICALS AND ANTI-INFLAMMATORY ACTIVITY OF *CAESALPINIA BONDUCELLA* (LINN)

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Received on: 30-03-2018; Revised and Accepted on: 02-05-2018

ABSTRACT

Inflammation is a kind of host defense against harmful stimuli like pathogens, irritations and damaged cells. The over production of inflammatory molecules can lead to several inflammatory diseases. Hence, it's essential to prevent the over expression of inflammatory molecules. Therefore the identification of the anti-inflammatory molecules is necessary and the medicinal plants are also used as a source of anti-inflammatory medicine in ancient practice. Hence, the present study was undertaken to study the phytochemical constituents, antioxidant and anti-inflammatory activity of ethanolic extract of leaf and seed kernel of Caesalpinia bonducella. The results revealed that, ethanolic extract of C. bonducella leaf and seed kernel showed the presence of rich amounts of various secondary metabolites like alkaloid, flavonoid, saponin, phytosterol and tannins. Moreover both leaf and seed kernel extracts exhibit potent antioxidant activity, which was evidenced by both leaf and seed kernel extracts potentially inhibited the formation of DPPH radicals. The anti-inflammatory activity results explored that both leaf and kernel extract of C. bonducella significantly inhibited the albumin denaturation, proteinase action as well as heat and hypotonic solution induced hemolysis of erythrocytes. Overall the results obtained in this study clearly demonstrated that, the ethanolic extract of both leaf and seed kernel of C. bonducella showed potent anti-inflammatory activity which was possessed may the presence of rich amount of various secondary metabolites.

KEYWORDS: *Caesalpinia bonducella, Anti-inflammatory, Secondary metabolites, Antioxidants.*

INTRODUCTION

Inflammation is a dynamic process that is elicited in response to mechanical injuries, burns, microbial infections and other noxious stimuli that may threaten the well-being of the host. This process involves changes in blood flow, increased vascular permeability, destruction of tissues via the activation and migration of leucocytes with synthesis of reactive oxygen derivatives and the synthesis of local inflammatory mediators such as prostaglandins (PGs), leukotrienes, and platelet-activating factors induced by phospholipase A2, cyclooxygenases (COXs), and lipoxygenases [1,2]. Inflammation localizes and eliminates microorganisms, damaged cells, and foreign particles, paving the way for a return to normal structure and function**.** The over accumulation of pro-inflammatory molecules like nitric oxide, TNF-α and interleukins-1β can decrease the macrophages activity and it can lead to pathogenesis of various chronic diseases like rheumatoid arthritis, atherosclerosis, diabetes and cancer [3]. Therefore inhibition of pro-inflammatory molecules is a possible route to reduce the effect of inflammation.

Since, the anti-inflammatory molecule from the natural sources is an active area of research to treat various kinds of inflammatory diseases. It has been estimated that in developed countries such as United States plant drugs constitute as much as 25% of the total drugs, while in fast developing countries such as India and China the contribution is as much as 80% [4]. India is a historical country in which plants play an important role with 45,000 medicinal plant sp. of which 3000 medicinal plants are documented and about 6000 plants are used by traditional practitioners [5].

Caesalpinia bonducella also called as 'grey nicer' is a species of flowering plant which reaches to a length of 6m and scrambles over other vegetation curved spines covers the stems. The plant has showed on various medicinal properties including febrifuge, antiperiodic, anthelmintic and tonic properties. The leaves juice or paste is used for relieving smallpox, elephantiasis, liver diseases and for eliminating bad odour in perspiration and for reducing toothache. Boiled leaves can be used for gargling to relieve sore throat. The seeds have astringent properties and are being used to relieve inflammation, contagious diseases, skin diseases hydrocele, colic and leprosy. The leaves and seeds after roasting in castor oil can be applied to reduce piles, inflammatory swellings, orchitis and hydrocele [6]. The wide range of medicinal properties of *C. bonducella* has taken on the account and the present study was decided to find out the phytochemical constituents and anti-inflammatory properties of ethanolic extract of leaf and seed kernel of *C. bonducella.*

MATERIALS AND METHODS

Collection and Authentication of plant materials:

The plant samples was collected from Siddha Medical college, Arumbakkam, Chennai and it was identified as *Caesalpinia bonducella* with the help of Flora of Presidency of Madras [7] and the flora of the Tamil Nadu and Carnatic [8]. The specimens were authenticated by **Prof. P. Jayaraman**, Director, PARC Research Center, West Tambaram Chennai, India (**Fig. 1**).

Preparation of crude extract:

The shade dried leaf and seed kernel was powdered with the help of mechanical pulverizer. The powdered material was then soaked in 1000 mL of ethanol. The extract was suction filtered using Whatmann filter paper. This was repeated for 2 to 3 times and similar extracts were pooled together and concentrated at 40 to 45°C under reduced pressure using vacuum rotary evaporator. The concentrated crude ethanolic

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DOI: https://doi.org/10.5281/zenodo.1249649

extract was subjected to a preliminary phytochemical analysis and biological activities [14].

Qualitative phytochemical screening:

The different qualitative phytochemical tests was performed for establishing the profile of given extract for its chemical composition. The tests for secondary metabolites were performed on the extracts to detect various phytoconstituents present in them.

Detection of alkaloids [9]
Detection of carbohydrates [10]
Detection of Saponins [11]
Detection of fixed oils and fats [11]
Detection of phenolic compounds and tannins [12]
Detection of Phytosterols [13]
Detection of Flavonoids [14]
Detection of terpenoids [10]
Detection of proteins and amino acids [15, 16]

Fig. 1: Entire picture of *Caesalpinia bonducella* (Linn.)
A - Entire shrub *C. bonducella* (Linn); B- Twig and Fruit of *C. bonducella* (Linn); C- Fruit of *C. bonducella* (Linn); D- Seed of *C. bonducella* (Linn)

Determination of total phenolic content:

The total phenolic content in the leaf and seed kernel of *C. bonducella* extracts were determined by Folin- ciocalteu method using Gallic acid standard [17]. To 1 mL of extract mixed with 1 mL of Folin-ciocalteu's phenol reagent (sigma). After 3 min incubation, one (1) mL of saturated sodium bicarbonate (3.5%) was added. The volume was made up to 10 mL with distilled water. The reaction mixture was kept in dark for 90 min. Absorbance was measured at 650 nm. The amount of total phenolic compound was calculated from the calibration curve of ascorbic acid standard solution. It was expressed as (GAE) Gallic acid equivalent in milligrams per gram extract.

$$C\ (GAE) = C \times V/M$$

Where, C=Concentration of sample from the curve obtained (mg/mL);
V=Volume used during the assay (mL);
M=Mass of the extract used during the assay (g).

Antioxidant Activity:

DPPH radical scavenging activity:

The antioxidant (free radical scavenging) activity of the partitionates on the stable radical 1,1-diphenyl- 2-picrylhydrazyl (DPPH) was determined by the method [18]. Here, 2.0 mg of each of the test sample was dissolved in ethanol and solution of varying concentrations was obtained by serial dilution technique. Then 2 mL of each of the test sample was mixed with 3 mL of a DPPH ethanol solution (20µg/mL) and was allowed to stand for 20 min for the reaction to occur. The absorbance was determined at 517 nm and from these values, the corresponding percentage of inhibitions were calculated by using the following equation:

$$\%\ inhibition = [1- (ABS\ sample / ABS\ control)] \times 100$$

Then % of inhibition was plotted against respective concentrations used and from the graph the IC_{50} was calculated using ascorbic acid, as the positive control. Three replicates of each sample were used for statistical analysis and the values are reported as mean ± SD.

GC-MS Profile of Caesalpinia bonducella (Linn.):

The Samples were processed by Gas Chromatography (GC) analysis was performed on an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID). For separation of FAMEs, a DB225 capillary column (30 m x 0.25 mm I.D.; 0.25 µm) was used. The initial oven temperature was maintained at 160°C for 2 min with a sequential increase to 180°C at 6°C min^{-1} for 2 min and 230°C at 4°C min^{-1}. The final oven temperature was maintained at 230°C for 10 min. Nitrogen was used as the carrier gas with a flow rate of 1.5 ml min^{-1}. The injector and FID temperatures were set at 230°C and 250°C respectively, while a split ratio of 50:1 was maintained for the analysis. The flame ionization detector allows for a large dynamic range and provides good sensitivity. Hydrogen is the carrier gas, nitrogen is the "makeup" gas, and air is used to support the flame.

The electronic signal from the GC detector is passed to the computer where the integration of peaks is performed. The electronic data is stored on the hard disk and the fatty acid methyl ester composition of the sample is compared to a stored database using the Sherlock pattern recognition software. All compounds are added quantitatively so that the gas chromatography performance may be evaluated by the software each time the calibration mixture is analyzed.

Assessment of *in vitro* anti-inflammatory activity:

Inhibition of albumin denaturation:

The anti-inflammatory activity of *Caesalpinia bonducella* was studied by using inhibition of albumin denaturation technique which was studied [19, 20] with minor modifications. The reaction mixture was consists of test extracts and 1% aqueous solution of bovine albumin fraction. The sample extracts were incubated at 37 ºC for 20 min and then heated to 51ºC for 20 min, after cooling the samples the turbidity was measured at 660nm. The Percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition = (Abs Control –Abs Sample) X 100/ Abs control

Anti-proteinase action:

The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mM Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 - 500 µg/ml). The mixture was incubated at 37°C for 5 min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210 nm against buffer as blank. The percentage inhibition of proteinase inhibitory activity was calculated [21].

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Membrane stabilization:

Preparation of Red Blood cells (RBCs) suspension:

The blood was collected from healthy human volunteer who has not taken any NSAIDs (Non Steroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline.

Heat induced haemolysis:

Different concentrations (100 - 500 µg/mL) of 1 mL test sample was added to 1 mL of 10% RBCs suspension was incubated in water bath at 56 ºC for 30 min with saline as control and Aspirin as a standard drug. All the centrifuge tubes containing reaction mixture were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The Percentage inhibition of Haemolysis was calculated as follows:

Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control

Hypotonicity-induced haemolysis:

Different concentration of extract (100-500µg/ml) 1 mL was mixed with 1 mL of phosphate buffer, 2 mL of hyposaline and 0.5 mL of HRBC suspension. Diclofenac sodium (100µg/mL) was used as a standard drug. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged at 3000 rpm. The supernatant was decanted and the haemoglobin content was estimated at 560nm.

Percentage protection = 100- (OD sample/OD control) x 100

RESULTS

Phytochemical analysis:

The ethanolic extraction of *C. bonducella* leaf and kernel extracts yielded 50-55% in solid- liquid ratio of 1:5. The preliminary phytochemical analysis of leaf and kernel ethanolic extracts of *C. bonducella* was estimated and the results showed the presence of alkaloids, saponin, phenolic, tannins, phytosterol and flavonoid compounds. Leaf extract showed the presence of carbohydrates whereas kernel did not respond to it, similarly kernel extract showed the presence of fixed oils and fats whereas the leaf did not respond to it. Moreover both leaf and kernel extract did not respond to Glycosides and Terpenoids **(Fig. 2).**

Comparative analysis of phenolic contents:

Phenols are the most diverse and widespread group of natural compounds. The total phenolic contents of crude ethanolic extracts of leaf and kernel extracts of *C. bonducella* were carried out. Phenol contents of *C. bonducella* were quantified and showed different range of phenolic content on leaf extract (2.260 mg/mL) and kernel extract (1.611 mg/mL) **(Table 1).**

DPPH radical scavenging activity:

Ethanol extracts of leaf and kernel showed various ranges of percentage of DPPH inhibitions. Among the two different parts, leaf extract showed maximum percentage of inhibition at 116.11 µg/mL (96.7%) followed by kernel extract showed 136.76 µg/mL (93.3%) whereas the standard ascorbic acid showed 60.14µg/mL. The better antioxidant activity was observed in leaf extract when compared with kernel extract **(Table 2).**

GC-MS Profile of Caesalpinia bonducella (Linn):

The GC-MS profile of *Caesalpinia bonducella* (Linn) showed the presence of various compounds in both leaf and kernel extract **(Table. 3 and 4; Fig. 3 and 4).**

Anti-inflammatory activity of ethanolic extracts of *C. bonducella:*

Inhibition of Albumin Denaturation:

The investigation on the mechanism of the anti-inflammation activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 44.68% was observed at 100 µg/ml in Kernel and 48.23% at 300µg/ml in leaf. Aspirin, a standard anti inflammation drug showed the maximum inhibition 68.15 % at the concentration of 100µg/ml compared with control **(Table 5).**

Proteinase Inhibitory Action:

Neutrophils are known to be a rich source of serine proteinase and are localized at lysosomes. *Caesalpinia bonducella* exhibited significant antiproteinase activity at different concentrations as shown in **(Table 6)**. It showed maximum inhibition of 52.5% at 500µg/ml in leaf extract where IC_{50} value was determined as 404.61µg/ml and 45% at 500µg/ml in kernel extract where IC_{50} value was determined as 555.55 µg/ml. Aspirin showed the maximum inhibition 55% at 100µg/ml.

Membrane stabilization:

Heat Induced Haemolysis:

The extract was effective in inhibiting the heat induced haemolysis at different concentrations. The results showed that *Caesalpinia bonducella* at concentration 400 and 500µg/ml protect significantly the erythrocyte membrane against lysis induced by heat (**Table 7**). The Inhibitory concentration of leaf and kernel extract was estimated as 326.22 µg/ml and 432.43 µg/ml. Aspirin 100µg/ml offered a significant protection against damaging effect of heat solution.

Hypotonicity Induced Haemolysis:

The results showed that *Caesalpinia bonducella* at concentration range of 100-500µg/ml protect significantly the erythrocyte membrane against lysis induced by hypotonic solution **(Table 8).** Diclofenac sodium (100µg/ml) offered a significant protection against the damaging effect of hypotonic solution. At the concentration of 500µg/ml, *Caesalpinia bonducella* showed maximum of 59.25% protection in leaf and 56.22% protection in kernel, whereas, Diclofenac sodium (100µg/ml) showed 51% inhibition of RBC haemolysis when compared with control.

Table No. 1: Estimation of Total Phenolic content in leaf and seed kernel ethanolic extracts of *C. bonducella*

Sample	Total Phenolic content (mg/mL)
Leaf	2.26 ± 0.39
Kernel	1.61 ± 0.19

Table No. 2: DPPH radical scavenging activity of leaf and seed kernel ethanolic extracts *C. bonducella*

S. No	Conc (µg/mL)	% of inhibition		
		Leaf	Kernel	Standard – Ascorbic acid
1	10	17.97±0.02	10.10±0.03	13.52±0.00
2	20	20.40±0.03	14.14±0.01	23.33±0.01
3	30	22.01±0.01	14.74±0.01	39.21±0.02
4	40	24.21±0.01	14.94±0.01	41.96±0.01
5	50	25.62±0.00	18.18±0.02	47.45±0.00
6	60	28.44±0.01	26.66±0.01	48.82±0.00
7	70	30.86±0.02	29.29±0.00	53.33±0.00
8	80	33.47±0.02	30.9±0.01	57.64±0.00
9	90	35.88±0.03	33.32±0.01	59.41±0.00
10	100	43.10±0.01	36.50±0.02	72.94±0.00

Table No. 3: GC-MS profile of *C. bonducella* leaf ethanolic extracts

Retention time	Compound Name	Chemical formula	Molecular weight
12.48	Anti -10-Methyl-endo- tricyclo[5.2.1.0(2.6)] decane	$C_{10}H_{16}$	136.23
16.02	1-cyclohexane-1- Methanol,4[1-methylethenyl]acetate	$C_{12}H_{18}O_2$	194.2701
16.45	Cyclopentanone, 2- Cyclopentylidene	$C_{10}H_{14}O$	150.2176
17.55	Benzofuran-2-one, 4-amino-2,3- dihydro	$C_8H_7NO_2$	149.1467
18.65	Phytol	$C_{20}H_{40}O$	296.539
19.15	Oleic Acid	$C_{18}H_{34}O_2$	282.47
22.57	Isopropyl Stearate	$C_{21}H_{42}O_2$	326.565
26.63	9,12,15-Octadecatrienoicacid, 2,3-dihydroxy propyl ester, (ZZZ)	$C_{27}H_{44}O_3$	352.261
28.5	Bufa-14,16,20,22tetraenolide, 3- (acetyloxy),(3a,5a)	$C_{26}H_{32}O_5$	418.52
31.68	17,[1,5-Dimethyl-hexyl]4,4,9,13,14-pentamethylhexadecahydrocyclopenta(a) phenanthren 3-one	$C_{30}H_{52}O$	428.7332

Table No. 4: GC-MS profile of *C. bonducella* seed kernel ethanolic extracts

Retention time	Compound Name	Chemical formula	Molecular weight
16.57	Flavone	$C_{15}H_{10}O_2$	222.24
17.58	Estra-1,3,5(10)trien-17 a-ol	$C_{20}H_{26}O_3$	314.42
19.1	Oleic Acid	$C_{18}H_{34}O_2$	282.47
26.07	Coumarine,3[2-[1-methyl-2-imidazolylthio]-1-oxoethyl]	$C_{15}H_{12}N_2O_3S$	300.3323
26.88	Isopropyl stearate	$C_{21}H_{42}O_2$	326.565
30.4	Benzoic acid, 4-hydroxy-3,5-dimethoxy-, octyl ester	$C_{17}H_{26}O_5$	310.39
35	2-Secoandrosta-1,4,6-triene-17,19-diol,2-cyano-4,methyl-diacetate	$C_{25}H_{33}NO_4$	411.542

Table No. 5: Effect of different concentration of leaf and kernel extracts of *C. bonducella* on the inhibition of albumin denaturation

S. No	Concentration of leaf and kernel extracts (µg/mL)	Inhibition of Protein denaturation (%)		IC_{50} value (µg/mL)	
		Leaf	Kernel	Leaf	Kernel
1	100	34.11	44.68	311.00	111.90
2	200	38.82	57.32		
3	300	48.23	66.52		
4	400	63.52	69.45		
5	500	70.58	74.05		

Table No. 6: Effect of different concentration of leaf and kernel extracts of *C. bonducella* on the inhibition of proteinase action

S. No	Concentration of leaf and kernel extracts (µg/mL)	Inhibition of proteinase action (%)		IC_{50} value (µg/mL)	
		Leaf	Kernel	Leaf	Kernel
1	100	15.30	15.44	404.61	555.55
2	200	24.20	22.63		
3	300	30.21	30.49		
4	400	49.43	37.15		
5	500	52.5	45.00		

Table No. 7: Effect of different concentration of leaf and kernel extracts of *C. bonducella* on heat induced haemolysis of erythrocytes

S. No	Concentration of leaf and kernel extracts (µg/mL)	Inhibition of Haemolysis (%)		IC_{50} value (µg/mL)	
		Leaf	Kernel	Leaf	Kernel
1	100	17.87	4.90	326.22	432.43
2	200	26.14	14.35		
3	300	45.98	37.79		
4	400	51.33	46.25		
5	500	56.06	54.50		

Table No. 8: Effect of different concentration of leaf and kernel extracts of *C. bonducella* on hypotonicity induced haemolysis of erythrocytes

S. No	Concentration of leaf and kernel extracts (µg/mL)	Inhibition of Haemolysis (%)		IC_{50} value (µg/mL)	
		Leaf	Kernel	Leaf	Kernel
1	100	15.52	07.46	419.55	444.68
2	200	28.15	18.46		
3	300	37.02	23.85		
4	400	47.67	33.81		
5	500	59.25	56.22		

Fig. 2: Different phytochemical analysis in leaf and seed kernel ethanolic extracts of *C. bonducella*
A – Alkaloids; B - Carbohydrate; C - Saponin; Phenols; E - Tannins; F - Phytosterols; G – Flavonoids; H - Fixed oils and Fats

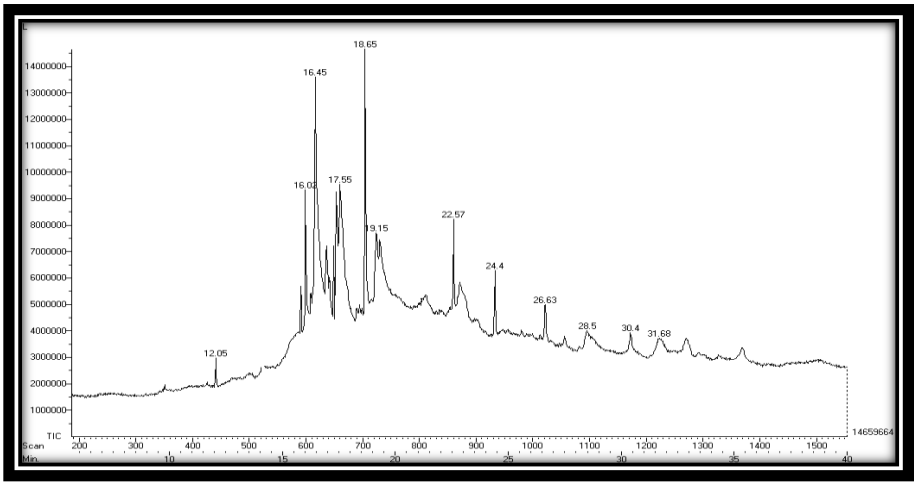

Fig. 3: GC-spectrum of *C. bonducella* leaf ethanolic extracts

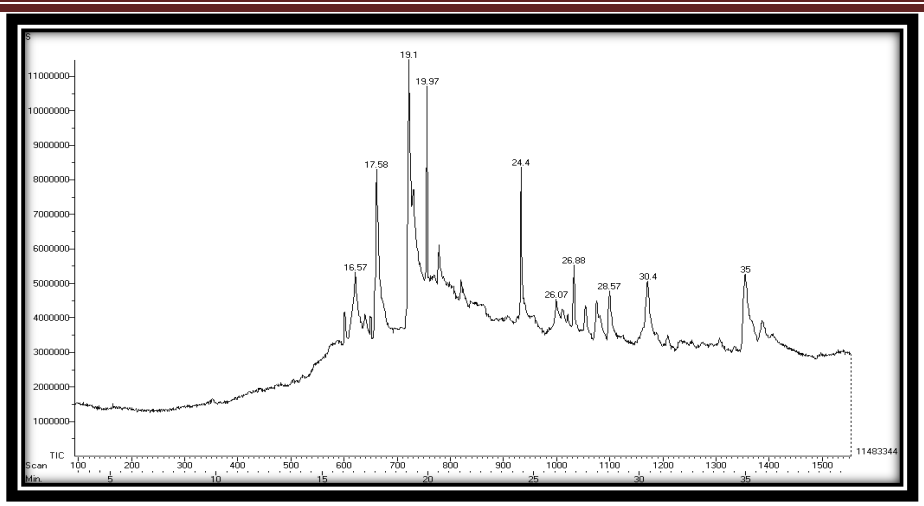

Fig. 4: GC-spectrum of *C. bonducella* seed kernel ethanolic extracts

DISCUSSIONS

Drug from natural product, especially from the medicinal plants is a emerging field to the treatment of various diseases. In general the herbal based drugs have excellent medicinal value and low toxicities and vast numbers of medicinal plants are used to treatment of several ailments. Moreover, in folk medicine, various indigenous medicinal plants are used in either single or in combined forms to treating of various types of inflammatory diseases [22]. In the present investigation the ethanolic extracts of both leaf and seed kernel of *C. bonducella* showed the presence of rich amount of secondary metabolites particularly high amount of phenolic content. In general plant polyphenolic compounds are considered as dietary antioxidants in human health and disease and it can protect the body tissues from oxidative damage by free radicals [23, 24]. The previous research has also indicated that phenolic compounds from the natural sources possessed vast number of biological application including antioxidant, anti-inflammatory and anticancer activity [25, 26]. Our study results correlated with the previous results on the whole plant of the *C. bonducella* contained all major secondary metabolites such as steroidal saponins, fatty acids, hydrocarbons, phytosterols, isoflavone, amino acids and phenolics [2, 5, 24].

The antioxidant potential of ethanolic extracts of both leaf and seed kernel of *C. bonducella* was evaluated by DPPH radical scavenging and the results explored that both extracts have potentially scavenge the formation of DPPH radical formation in a dose dependent manner with the IC_{50} value of ~ 60 µg/ml for both leaf and kernel extract respectively. In these contrast, the chloroform extract of seeds of *C. bonducella* showed effective antioxidant activity with IC_{50} value of 170±4.08µg/mL and the antioxidant activity may possessed by the presence of high amount of phenolic content (21.96 ± 2.12 GAE) [27].

The GC-MS analysis of *C. bonducella* leaf and kernel showed the presence of various phytoconstituents. Likewise in previous study the petroleum ether fraction of *C. bonducella* subjected to GC-MS analysis showed the presence of various phytochemical compounds from which 3 cyclic compounds were identified as 1H-Naphtho[2,1-b]pyran,3-ethenyl dodecahydro-3,4a,7,7,10a-penta methyl [28]. The GC-MS results proved the presence of various phytochemicals in both leaf and kernel extract of *C. bonducella* and it can responsible for the various biological activity of these plant extracts.

Generally protein has lost their structure during oxidative stress from the external stress like heat. Moreover most of the protein has also lost their biological functions when it was denatured. The denatured protein not only lost their biological functions it can also lead to generate many inflammatory diseases [29]. Hence, the inhibitory effect of *C. bonducella* leaf and kernel extracts was evaluated on heat induced albumin danaturation. The results revealed that both leaf and kernel has significantly reduced the heat induced protein denaturation. Similarly [29] Leelaprakash and Mohan Dass (2011) reported that, the methanolic extract of *Enicostemma axillare* has potentially inhibited the protein denaturation up to 71% at 500 µg/mL concentration.

The enzyme serine protease abundantly present in the lysosomes of the neutrophils and it can damage many kind of cells during inflammation [30]. The present study results clearly indicated that the ethanolic extracts of both leaf and seed kernel of *C. bonducella* showed potent antiproteinase activity by inhibiting the proteinase action. In these contrast the methanolic extracts of *Acer platanoides* significantly inhibited the action of proteinase at the IC_{50} value of 1.8µg/Ml [31].

The membrane stabilization of human red blood cells (HRBC) has been used as a method to investigate the *in vitro* anti-inflammatory activity because the membrane of erythrocyte is similar to the lysosomal membrane. Hence, the stabilization of erythrocyte membrane is implicating the stabilization of lysosomal membranes. The lysosomal membrane stabilization is an important for reduce the inflammatory response by preventing the release of lysosomal constituents of activated neutrophil [32, 33]. Our present investigation obviously indicated that the both leaf and kernel ethanolic extracts of *C. bonducella* probably reduced the heat and hypotonicity solution induced hemolysis of HRBC and explored the erythrocytes membrane stabilization process. Our study results correlated with [34] Anosike et al. (2012) reported that the methanolic extract of *Solanum aethiopicum* significantly reduced the heat and hypotonicity solution induced hemolysis of HRBC up to 86.67 ± 3.06 and 50.8 ± 3.75% respectively at 800 µg/mL concentration.

CONCLUSION

Collectively, the present study results concluded that the ethanolic extracts of *Caesalpinia boducella* possessed potent antioxidant and anti-inflammatory activity. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenols. This study gives on idea that the compound of the plant *C. boducella* can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases inflammatory diseases.

ACKNOWLEDGEMENT

Our Sincere thanks to Professor **P. Jayaraman** Director, PARC Research Center, West Tambaram Chennai, India and Authorities of **University of Madras** for providing the facilities to carry out the research work.

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How to cite this article:

S. Elumalai et al. STUDIES ON PHYTOCHEMICALS AND ANTI-INFLAMMATORY ACTIVITY OF CAESALPINIA BONDUCELLA (LINN). J Pharm Res 2018;7(5):63-69. **DOI: https://doi.org/10.5281/zenodo.1249649**

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nil