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In vivo anti-inflammatory activity and GC-MS analysis of hydroethanolic extract of *Caesalpinia bonducella* seeds

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Abstract
Objective: To investigate the GC-MS analysis and anti-inflammatory activity of hydroethanolic extract of *C. bonducella* seeds in wistar rats.
Methods: The acute anti-inflammatory activity was studied by Carrageenan induced rat paw edema while chronic anti-inflammatory activity by cotton pellet induced granuloma model.
Results: The GC-MS analysis showed presence of 32 phytoconstituents. Among these 16 compounds which were previously reported for the presence of various biological activities. From the study, it was shown that the extract significantly inhibited paw volume in acute inflammation and weight of granuloma formation in chronic inflammation at both the doses in dose dependent manner.
Conclusion: From the results obtained, it was concluded that the *C. bonducella* extract possesses significant anti-inflammatory activity which validated its ethnopharmacological use in treatment of inflammation.

Keywords: Anti-inflammatory activity, GC-MS analysis, *Caesalpinia bonducella*, hydroethanolic extract

Introduction
Herbal medicines and alternative medicines are being used throughout the world. In the past, herbs were the main source of most of the drugs (Saad *et al.*, 2005) [1]. Plants are popularly known for its medicinal values and these phytomedicines have great promise in treatment of various animal and human diseases. Although a large number of plant species are available worldwide, only marginal amount species of plants are analyzed phytochemically and pharmacologically to be used as a medicine (Gbenou *et al.*, 2013) [2]. According to WHO, approximately 75-80% of world's population especially in developing countries relies on plants for primary health care because of poverty and less access to modern medicine (Calixto, 2000) [3].
Inflammation is the reaction of vascular and supporting element of tissues to injurious stimuli. Thesestimuli include mechanical, radiations, extreme temperature, ischemia, infectious and immunological agents. The redness, swelling, heat, pain and loss of function are the cardinal signs of inflammation at microscopic level (Emamuzo *et al.*, 2010) [4]. The drugs available for the treatment ofinflammation and pyrexia are NSAIDs. These drugs have a wide range of side effects due to non-selective inhibition of COX-I and COX-II (Ahmadiani *et al.*, 2001) [5]. The three species of Gyps vultures are on verge of extinction due to exposure to diclofenac when they consume the carcass of livestock treated with diclofenac shortly before death. Apart from Gyps, NSAIDs are also harmful to raptors, storks, cranes and owls (Cuthbert *et al.*, 2007) [6]. So many studies are being taken to discover drugs which selectively inhibit COX-II or with other mechanism with little side effects (Ahmadiani *et al.*, 2001) [5].
Caesalpinia bonducella is a prickly shrub belonging to family *Fabaceae/ Caesalpiniaceae* distributed all over the world. The word bonducella is taken from Arabic word '*bonduce*' which means little ball that indicates shape of the seeds (Devi *et al.*, 2016) [7]. It is very valuable plant which is widely used in traditional medicine because all parts of this medicinal plant have reported medicinal properties (Singh and Raghav, 2012) [8].
So the present study is aimed at investigating the anti-inflammatory effect of seeds of *Caesalpinia bonducella* as a step to scientifically revalidate it's folkloric use.

2. Material and method
2.1. Experimental Animals
The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC). The Wistar rats (150-250 gm) of equal sex ratio were used in this study. The rats were

procured from National Institute of Biosciences, Pune. All the rats were reared under standard manage mental conditions as per the norms of CPCSEA.

2.2. Plant Material
The seeds of *Caesalpinia bonducella* were collected from Cattle Breeding Farm, Nagpur Veterinary College, Nagpur. The plantwas duly authenticated from RTM University, Nagpur.

2.3. Preparation of extract:
The seeds were air dried at room temperature and powdered. The powder was initially subjected to defattening with n-Hexane in Soxhlet's apparatus. The defattened powder was dried and subsequently extracted with 70% ethanol in Soxhlet's apparatus. The extract was made solvent free by air drying. The obtained extract was semisolid brown in colour with percent extractability of 12.32%. The preliminary phytochemical analysis revealed presence of Alkaloids, Carbohydrates, Phytosterols, Phenolic compounds and Flavonoids.

2.4. GC-MS analysis of hydroethanolic seed extract:
The GC-MS analysis of Hydroethanolic extract of seeds of *C. bonducella* was carried out using Agilent GC 7890 with triple axis 5975 MS detector. The capillary column was Agilient HP-5MS (30 m x 250 µm x 0.25 µm) composed of 5% phenyl methyl silox. The initial oven temperature was 40^0C for 0 min which was raised at rate of 25^0C/min upto 160^0C for 15 min and then at rate of 2^0C/min upto 280^0C for the hold time of 5 min. The injector volume was 4 µl.
The Helium gas used as carrier with constant flow rate of 1 ml/min with split ratio of 25:1. The MS operating conditions were; source temperature 230^0C (max-250^0C), quad temperature 150^0C (max-200^0C), solvent delay time of 4 min. Compounds were identified in terms of Rt values and mass spectra with those obtained from the NIST search library. The obtained compounds were searched for detailed pharmacological activities

2.5. Acute Oral Toxicity test
Acute oral toxicity study of hydro-ethanolic extract of *Caesalpinia bonducella* seed was carried out in female Wistar rats by following OECD guidelines, Test No- 423. A limit test at one dose level of 2000 mg/kg body weight was carried out with six animals (3 animals per step). The animals were fasted overnight and were administered with seed extract at dose rate of 2000 mg/kg body weight. The animals were observed for the appearance of any toxicity symptoms.

2.6. Anti-inflammatory activity
The hydroethanolic extract of seeds of *Caesalpinia bonducella* was screened for acute anti-inflammatory activity by carrageenan induced rat paw edema (Ishola *et al.*, 2011) [9] and cotton pellet induced granuloma model (Ramprasath *et al.*, 2004) [10] was used for chronic anti-inflammatory activity.

2.6.1. Carrageenan induced rat paw edema (Acute Inflammation):
The initial paw volume of right hind limb of each rat was measured by method described by Patgiri, *et al.*, 2014 [11] with some modifications. Edema was produced by injecting 0.1 ml of freshly prepared 1% Carrageenan, w/v in saline into sub-plantar tissue of right hind paw one hour after drug administration. The paw volume was measured at 1 hour interval for 5 hours and percent inhibition was calculated by the formula:

% inhibition= [increase in paw volume (control) - increase in paw volume (treatment)/ increase in paw volume (control)] x 100

2.6.2. Cotton pellet induced granuloma (Chronic Inflammation)
Thirty minutes after administration of drugs, the rats were anaesthetized by Xylazine (8 mg/kg body weight) and Ketamine (60 mg/kg body weight). The cotton pellets of 20 mg each were measured and sterilized in hot air oven. Cotton pellets were implanted at interscapular depth under skin. Animals were treated with drugs daily for 7 consecutive days. On 8^{th} day, rats were again anaesthetized and the pellets and surrounding granulomatous tissues were removed, immediately weighed and dried for 24 hrs at 60^OC temperature in hot air oven and again weighed. The difference between dry and wet weights of pellet was taken as measure of Granuloma formation which was compared with the control and percent inhibition was calculated:

% inhibition= [weight of cotton pellet (control) - weight of cotton pellet (treatment) / weight of cotton pellet (control)] x 100

2.7. Statistical analysis
All the values were represented as Mean±SE. The statistical differences between means of treatments were evaluated by completely randomized design (CRD) for chronic anti-inflammatory activity. While acute anti-inflammatory activity was statistically analyzed by ANOVA: two factors with replication. A 'P' value less than 5% was considered as statistically significant ($P<0.05$).

3. Results
3.1. GC-MS analysis
The hydroethanolic extract of *Caesalpinia bonducella* seed was subjected to GC-MS analysis. The compounds found in the GC-MS analysis with their retention time, peak area and % area is presented in table no. 1. The detailed chromatogram showing peaks of identified phytochemical is shown in graph no.1.

3.2. Acute Oral Toxicity
The acute oral toxicity was performed as per the OECD guideline no. 423. The toxicity study was carried out in three female rats at the dose rate of 2000 mg/kg body weight. There was no mortality and no one rat showed any sign of physiological and behavioral changes during the observation period of 14 days.

3.3. Anti-inflammatory Study
3.3.1. Carrageenan induced rat paw edema (Acute inflammation)
The effect of test drug on carrageenan induced acute inflammation is summarized in table no. 3.The sub-plantar injection of 0.1 ml of freshly prepared carrageenan significantly produced paw edema in control group. Both the standard drug and test drugs significantly decreased the paw volume. The test drug *C. bonducella* hydroethanolic extract at the dose of 200 &400 mg/kg showed significant inhibition of paw edema in dose dependent manner.

3.3.2. Cotton pellet induced granuloma (Chronic inflammation)

The wet weight, dry weight and granulomatous tissue formed in control, standard and two test groups are depicted in table no. 4. From that, it can be noted that the standard drug indomethacin and two doses of *C. bonducella* hydroethanolic extract significantly reduced the wet weight, dry weight and weight of granulomatous tissue formed.

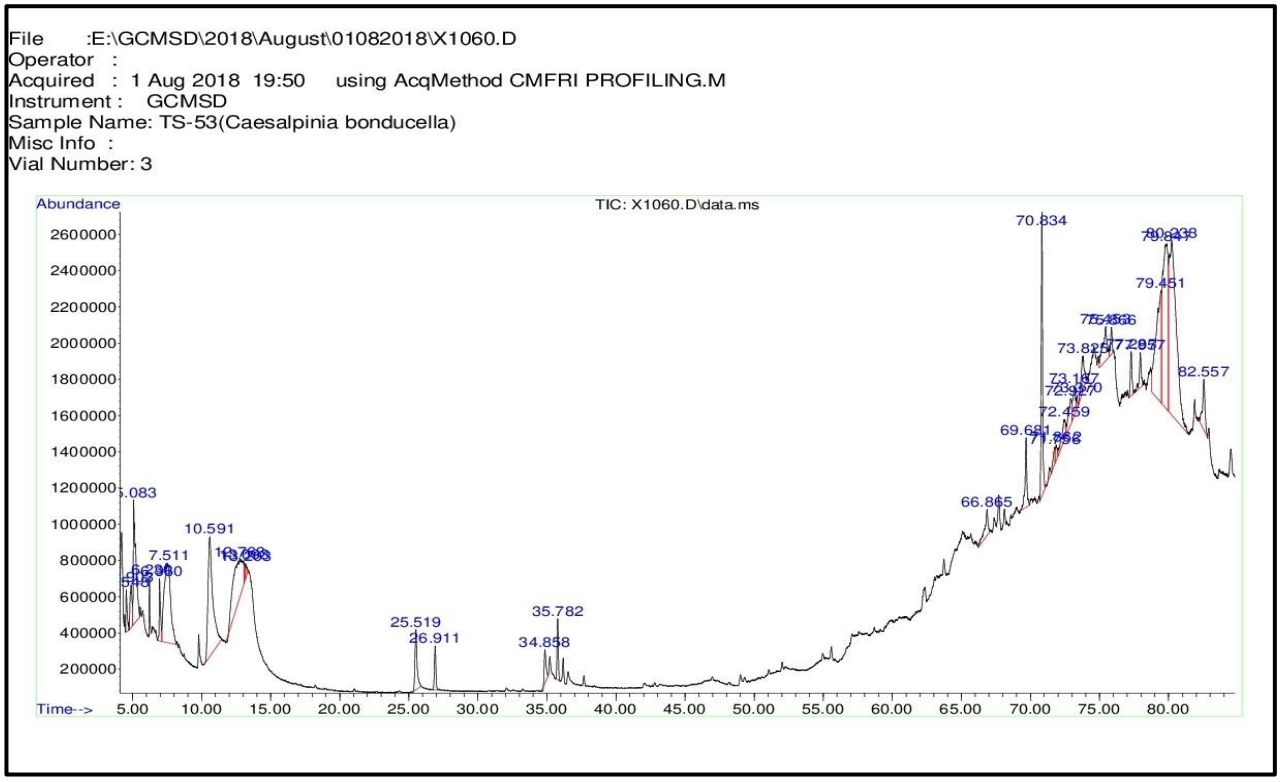
Table 1: Showing identified compound in the GC-MS analysis of hydroethanolic extract of *C. bonducella* seed extract with their retention time, peak area and % area.

Sr. No.	Identified compound	R. T. (min)	Peak area	% area
1	2,3, dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran-4-one	4.54	229215	0.759
2	Pentanoic acid	4.90	232231	1.22
3	2-Pyrazoline	5.08	681492	4.707
4	2-Propanol	6.23	291284	0.415
5	1-Piperidineethanol 2-Propenoic acid	6.96	346569	1.18
6	1-Propanamine	7.5	438699	7.79
7	Ethyl. beta -d-riboside	10.59	660881	8.61
8	3-O-Methyl-d-glucose.alpha.-D-Xylofuranoside	12.76	208892	6.24
9	2-[2-(2-Ethoxyethoxy)ethoxy]ethyl acetate	13.09	111469	0.354
10	Hydrazine	13.20	75580	0.23
11	n-Hexadecanoic acid	25.51	330932	1.89
12	Hexadecanoic acid ethyl ester	26.91	240598	0.95
13	9,12-Octadecadienoic acid (Z,Z)	34.85	184367	0.854
14	Linoleic acid ethyl ester	35.78	337199	1.16
15	Trimethylsilyl ether	66.86	143483	1.26
16	9-Octadecene	69.68	378918	1.90
17	1-(4-Nitrophenyl)-3-phenyl-3,4-di(carboxyethyl)-pyrazolin Acetic acid	70.83	1567068	5.95
18	2,3-Dihydroinden-2-one	71.15	93798	0.65
19	1H-Imidazole	71.86	82978	0.42
20	Dichotine	72.45	116119	1.22
21	Ethanone	72.92	140358	1.32
22	2,2'-(Alpha-methylbenzylidene) bis(6-methoxy-3-methylbenzofuran)	73.16	163922	1.17
23	1,1-dimethoxy Ethanone	73.37	75266	0.203
24	2,3-Dihydroinden-2-one	73.82	184574	1.69
25	2-Furanmethanamine	75.45	187917	2.18
26	Tetrahydro-Nalmefene	75.86	145456	0.626
27	o-(3-methylbutyl)-9-octadecene	77.28	244976	1.10
28	Hydroxylamine	77.95	191964	1.01
29	Tetrahydro- Dichotine	79.45	619972	8.493
30	1-(Trihexylsilyloxy) tetradecane Boron	79.84	910879	13.38
31	1,2,3-Thiadiazole	80.23	962848	19.08
32	tetrahydro-Quinoline	82.55	278011	1.88

Table 2: Earlier reported biological activities of phytochemical identified in the GC-MS analysis of hydroethanolic extracts of seeds of *Caesalpinia bonducella.*

Sr No.	Identified compounds	Pharmacological activities
1	2,3, dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran-4-one	Antimicrobial, Anti-inflammatory, Antiproliferative, Antioxidant, Automatic nerve activity (Gopalkrishnan and Udayakumar, 2014) [12]
2	2-Pyrazoline	Anti-microbial, anti-mycobacterial, antiamoebic, analgesic, anti-inflammatory, anti-convulsant, cytotoxic anti-depressant, hypotensive, cytotoxic, anticancer, anti-oxidant, ACE inhibitor (Bardalai and Paneerselvam, 2012) [13]
3	n-Hexadecanoic acid	Anti-inflammatory, Antioxidant, Hypocholesterolaemia, nematicide, pesticide, antiandrogenicflavor, hemolytic,5-Alpha reductase inhibitor, potent mosquito larvacidal (Abubkar and Majinda, 2016) [14]
4	Hexadecanoic acid ethyl ester	Antioxidant, HypocholesterolemicNematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor (Rajeswari *et al.*, 2012) [15] anti-inflammatory (Swamy *et al.*, 2015) [16]
5	9,12-Octadecadienoic acid (Z,Z)/	Anti-inflammatory, Nematicide, Insectifuge, Antiacne, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiarthritic, Antieczemic,5-Alpha reductase inhibitor, Antiandrogenic, Anticoronary (Sermakkani and Thangapandian,2012) [17]
6	Linoleic acid ethyl ester	Hypocholesterolemic, Nematicide, Anti-acne, antiarthritic, Hepatoprotective, Anti-androgenic, Hypocholesterolemic, 5-Alpha Reductase inhibitor, Antihistaminic, Anticoronary, Insectifuge, Anti-eczemic (Tyagi and Agarwal, 2017) [18]
7	Trimethylsilyl ether	Antitumor (Abdelhamid *et al.*, 2015) [19]
8	hydrazine	Antimicrobial and antifeedant (Goff and Ouazzani, 2014) [20]
9	3-O-Methyl-d-glucose	Preservatives (Sermakkani and Thangapandian,2012) [17]
10	Pentanoic acid	Potential biofuels can be prepared. Also used in cigarettes to increase nicotine delivery in smoke and binding of nicotine to neural receptors (Jadhav *et al.*, 2014) [21]
11	2- propanol	Antibacterial, antiseptic, anesthetic, surfactant, neurolytic (Karthika *et al.*, 2013) [22]
12	2-Furanmethanamine	Industrially used as intermediates (Marandi *et al.*, 2018) [23]
13	1-propanamine	Antifungal, antiviral (Gunalan *et al.*, 2014) [24]

14	1-piperidineethanol	Alleviating the problem of loss of appetite (Perumal *et al.*, 2018) [25]
15	1,2,3-thiadiazole	Fungicidal (Fan *et al.*, 2009) [26]
16	1H-Imidazole derivatives	Antifungal, antibacterial, anti-inflammatory, analgesic, antidepressant, antitubercular, antitumor, antiviral and antileishmanial (Abbasov *et al.*, 2012) [27]



Graph 1: Showing chromatograph

Table 3: Effect of ethanolic extract of *Caesalpinia bonducella* seeds on Carrageenan induced paw edema in rats.

Groups	Treatment	Initial Paw Volume	Increase in paw volume after treatment (sec)				
			1 hr	2 hr	3 hr	4 hr	5 hr
T_1	Normal saline	1.184±0.05	1.58^{b}±0.06^{A}	1.81^{b}±0.08^{A}	1.9^{d}±0.07^{A}	1.83^{d}±0.07^{A}	1.65^{d}±0.07^{A}
T_2	Indomethacin @ 10 mg/kg	1.31±0.04	1.44^{a}±0.08^{A} (67.5%)	1.54^{a}±0.04^{A} (63.49%)	1.45a±0.05A (80.55%)	1.41^{a}±0. 03^{A} (84.61%)	1.35^{a}±0.03^{A} (91.48%)
T_3	*C. bonducella* @ 200 mg/kg	1.31±0.08	1.66^{c}±0.07^{A} (12.5%)	1.76^{b}±0.08^{A} (28.57%)	1.68^{c}±0.1^{A} (48.6%)	1.61^{c}±0.1^{A} (53.84%)	1.55^{c}±0.08^{A} (48.93%)
T_4	*C. bonducella* @ 400 mg/kg	1.26±0.06	1.55^{b}±0.06^{A} (27.5%)	1.61^{a}±0.08^{A} (44.45%)	1.53^{b}±0.1^{A} (62.5%)	1.50^{b}±0.1^{A} (63.07%)	1.43^{b}±0.1^{A} (63.82%)

Each value is the mean±SE of six rats. C. D. value for treatment= 0.08
Values in the bracket indicate the percent inhibition. C. D. value for hours= 0.19
All values are significant at 5% level of significance.

Table 4: Effect of ethanolic extract of *Caesalpinia bonducella* seeds on Cotton pellet induced granuloma model in rats.

Sr. No.	Groups	Treatments	Wet weight (mg)	Dry weight (mg)	Granuloma formation (mg)
1	T_1	Normal saline	424.34^{d}±9.32	91.8^{c}±3.27	71.8^{c}±3.27
2	T_2	Indomethacin @ 10 mg/kg	175.33^{a}±9.40 (58.68%)	51.16^{a}±5.85 (44.27 %)	31.16^{a}±5.85 (56.60%)
3	T_3	*C. bonducella* @ 200 mg/kg	366.34^{c}±13.6 (13.66%)	78.75^{b}±3.99 (14.21%)	58.75^{b}±3.99 (18.17%)
4	T_4	*C. bonducella* @ 400 mg/kg	287.5^{b}±7.89 (32.24%)	70.234^{b}±2.26 (23.49%)	50.234^{b}±2.2 (30.03%)

Each value is the mean±SE of six rats. C. D. value = 9.19
Values in the bracket indicate the percent inhibition.
All values are significant at 5% level of significance

Discussion

Carrageenan is a widely used agent for experimental induction of inflammation to screen the compound having anti-inflammatory activity. This agent when injected locally produces severe inflammatory condition which is noticeable within 30 min (Bhandare *et al.*, 2010) [28]. As typically observed in acute inflammation, the carrageenan induces inflammation in 2 phases. The phase 1 is early phase and lasts upto 2.5 Hrs after carrageenan injection that is characterized by liberation of serotonin, bradykinin and histamine, while the late phase is characterized by release of prostaglandins and other inflammatory mediators (Zhu *et al.*, 2011) [29]. In present study hydroethanolic extract of *C. bonducella* at all the dose levels significantly inhibited both the phases of carrageenan induced rat paw edema, which may indicates that the extract may have non-selectively inhibited some of these inflammatory mediators. It was observed that, extract was more efficacious from 3 Hr in reducing the paw edema which

indicates that the extract might be inhibiting the prostaglandins.

The cotton pellet induced granuloma for chronic inflammation has three phases: transudative phase for first 3 Hr, exudative phase from 3 to 72 Hr of implantation of cotton pellet and third and most important is proliferative phase which increases the dry weight of cotton pellet from third day to sixth day (Ambekar *et al.,* 2011) [30]. For evaluation of transudative and proliferative component of chronic inflammation, cotton pellet granuloma is mostly used. The wet weight represents the transudative material while dry weight represents the granuloma tissue formed (Babu and Karki, 2011) [31]. Chronic inflammation is characterized by monocytes infiltration and fibroblast proliferation rather than neutrophil infiltration and exudation (Suleyman *et al.,* 2007) [32]. NSAIDs inhibits granulocyte infiltration, prevent generation of collagen fibres and suppress mucopolysaccharides and thus decreases the size of granuloma (Ramprasath *et al.,* 2004). As the hydroethanolic extract reduces the weight of wet and dry granuloma, the extract may have exhibited the anti-inflammatory activity by inhibiting granulocyte infiltration and fibroblast infiltration. Arunadevi *et al.,* 2015 [33] reported significant acute and chronic anti-inflammatory activity of *C. bonducella* flowers in rats in carrageenan induced rat paw edema model and cotton pellet induced granuloma model and mentioned that the plant flavonoids suppresses inflammatory mediators like TNFα, PGs and NO along with inducible COX and NO synthase enzyme.

The phytochemical studies revealed the presence of flavonoids qualitatively in the extract of *C. bonducella* seeds, which must be contributing in suppression of some of the inflammatory mediators responsible for maintenance of acute and chronic inflammatory responses. In present study the GC-MS analysis revealed presence of compound 2,3, dihydro-3, 5-dihydroxy-6-methyl-4H-Pyran-4-one, 2-Pyrazoline, 9,12-octadecadienoic acid, n-hexadecanoic acid, linoliec acid ethyl ester, hexadecanoic acid and 1H-Imidazole compounds which were earlier attributed for demonstration of anti-inflammatory activity. Therefore in general the acute and chronic anti-inflammatory activity as shown by *C. bonducella* seeds extract in present study may be attributed for the presence of flavonoids and other bioactive phytoconstituents which are may be responsible to inhibit the major mediators in the inflammatory pathway.

5. Conclusion(s)

From the results obtained, it is concluded that the whole seed of *C. bonducella* containing seed coat and seed kernel is having significant anti-inflammatory activities. The detailed phytochemical analysis of *C. bonducella* seed extract with Gas Chromatography and Mass Spectrometry (GC-MS) revealed presence of 32 bioactive compounds some of which are reported earlier for exhibition of pharmacological activities. Therefore, further studies are essential to find out the mechanism of action(s) responsible for demonstration of pharmacological activities by the *C. bonducella* seeds.

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