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Original Article

Finding the Principle Leads using GC-MS and Unravelling the Anti-inflammatory Activity of Alkaloid Isolated from *Caesalpinia bonducella* by *in vitro* Techniques

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ABSTRACT

Aim/Background: Plant-based medicines have gained remarkable recognition recently, as consumers have begun to express their preference for herbal remedies. *C. bonducella*, commonly known as fever nut, is said to have massive value in Ayurveda regarding health and cosmetics. We aim to envisage the alkaloid for its anti-inflammatory property using *in-vitro* techniques. **Materials and Methods:** *C. bonducella* powder, soxhlet apparatus, solvents including hexane, DCM, ethyl acetate and methanol. GC-MS, L929 cells, DMEM medium, antibiotic solution, sulfanilamide, naphthylethylenediamine, phosphoric acid, ferric chloride, sodium hydroxide, potassium iodide and ammonium hydroxide. **Results:** The cell-free *in-vitro* anti-inflammatory assay showed 75% Membrane Stabilization, 125% Albumin denaturation assay and 106% Proteinase inhibition assay at 1mg/mL of aqueous extract. The cell viability remained unaltered even in the highest tested concentration, with a dose-dependent nitrite inhibition count recorded. **Conclusion:** Alkaloids, carbohydrates and flavonoids showed their presence in aqueous solution of seed powder. This study showed that the isolated alkaloids found to be in higher concentration in Aqueous extract and subsequently employs GC-MS to understand the various compounds present in it. The attenuation of pro-inflammatory mediator Nitric oxide was significant in Aqueous extract which supports the anti-inflammatory potential of the extract.

Keywords: *Caesalpinia bonducella*, Fever nut seeds, GC-MS, Anti-inflammatory, Alkaloid.

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INTRODUCTION

Inflammatory responses stimulated by the immune system act as a defense mechanism against the foreign agent, such as microorganisms, dust particles, and damaged tissues or cells.[1] This mechanism of the body causes redness, pain and swelling in the infected region because of changes or remodeling of the membrane where protein denaturation increases along with vascular permeability.[2]

Caesalpinia bonducella has been employed as a medicinal herb for ages for the treatment of different diseases because of its high nutraceutical value. The leaves of this prickly shrub have been studied extensively for curing numerous diseases namely, piles, inflammatory swellings, and orchitis.[3]

A human Red Blood Cell Membrane Stabilization assay is conducted since HRBCs mimic the lysosomal membrane. So, stabilization of the former will stabilize the lysosomal membrane, thereby protecting the membrane from degrading at the time of inflammation.[4] It is also studied that neutrophils have an abundant amount of serine proteinase and leukocyte proteinase, which mediates the development of tissue damage at the time of inflammation. Neutrophils are those phagocytic cells which has abundant levels of lysozyme (an enzyme secreted by lysosomes) which secretes serine proteinase/protease that kills the bacteria. But, over secretion of these proteases may lead to the damage of the tissues microenvironment, causing inflammation. So, the seed for anti-proteinase activity has been studied to provide significant levels of protection.[5] Moreover, proteins are known to lose their biological function during inflammation due to modification in their secondary/ tertiary structure. Alongside, It is also seen that nitric oxide is released in high amounts during injury, which further leads to tissue damage when conjugated with Superoxide radical. Metabolically, nitrates are converted to nitrites in our bodies. Generally, the nitrates and nitrites are absorbed by the stomach and small intestine after they are ingested. These nitrates are immediately circulated all over the body internally through plasma after accurately binding with RBCs/erythrocytes. The lower part of the small intestine also provides a way for nitrate introduction into our body. Furthermore, these get converted to reactive nitrite ions once the metabolism is complete when

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gut bacteria act on them. The reactive nitrite ions get absorbed again by the small intestine, which further converts them to methemoglobin after reacting with ferrous ions present in haemoglobin. This scenario is only observed if the conditions of the small intestine are suitable for reducing nitrate to nitrite, which includes high pH and a specific amount of microbiota in the intestine. If the conditions are not favourable, the nitrate gets excreted through urine after ingestion without causing side effects or accumulating in nearby tissues. Lipopolysaccharide (LPS) has a significant part in inducing inflammatory responses that cause various diseases and disorders. LPS is an extracellular component of gram-negative bacteria that is capable of interacting with certain receptors present on host effector cells. This further acts as a mediator and leads to the expression of pro-inflammatory cytokines. Immune cells such as Macrophagic cell line are capable of portraying as a suitable model for *in vitro* Inflammatory cells that perform pinocytosis and release Nitric Oxide (NO). The same cells are also capable of killing target cells via antibody-dependent cytotoxicity.

So, in this study, we conduct a thorough qualitative and quantitative analysis of the seed powder and focus on the anti-inflammatory effects of the isolated and purified bioactive leads from it.

MATERIALS AND METHODS

Source of the *C. bonducella* seeds

The *C. bonducella* seeds were purchased from the local drug store.

Preparation of Plant Extract

C. bonducella seeds (kernels) were obtained. Further, they were shade dried for 15 days. The seeds were finely powdered and kept in an airtight container.[4]

Sequential Extraction

For sequential extraction using various solvents, including hexane, DCM, EA, methanol, and aqueous solutions, 50 g of the finely powdered seed was taken in a pouch made of Whatman filter paper in the thimble of the soxhlet apparatus (Figure 1). These solvents were poured through the condenser inlet, each measuring 100 mL, except DCM, which measured 300 mL because of its volatile nature. Soxhlet apparatus was left to run for 6-7 hr at 69°C. The gathered solvents were poured into beakers and covered with aluminium foil. The foils were punched with holes and left overnight. Additionally, the solvents have been concentrated for roughly 15 min in a rotary vacuum evaporator operating at reduced pressure and 45°C. The extracts were weighed and kept in petri dishes for 5-6 days under sun drying.[4]

The yield of various extracts

The extract yield was determined using the formula:

The Yield percentage (%y) = {weight of the powdered sample obtained (g)/ weight of the sample extract utilized (g)* 100

In vitro anti-inflammatory assays

Albumin denaturation assay

The control tube consisted of 1 mL BSA and 1 mL Millipore water. 1 mL BSA was added in different test tubes containing varying concentrations (100 µg, 200 µg, 400 µg, 600 µg, 800 µg and 1000 µg) of plant extract. Aspirin was taken as the standard drug, and 1 mL was added to a test tube along with 1 mL BSA. The test tube stand was placed in an incubator for 15 min at 37°C and then for 20 min at 51°C. The tubes were cooled and placed in running water. UV-vis spectrophotometer was utilized to record the absorbance at 660 nm. Inhibition potential for albumin denaturation was determined by the formula given below:

% Inhibition = {Absorbance of control/Absorbance of test} * 100

Proteinase Inhibition assay

The reaction mixture consisted of 1 mL 20 mM Tris HCl buffer calibrated at pH 7.4, 0.06 mg trypsin and 1 mL plant extracts. The mixture was kept in an incubator for 5 min at 37°C. Further 1 mL 0.8% (w/v) casein was added to the mixture. The reaction mix was put for incubation at 37°C for 20 min. Next, reaction termination was achieved by adding 2 mL of 70% perchloric acid. Lastly, the tube containing the mixture was vortexed. UV-vis spectrophotometer was utilized to record the absorbance of the supernatant at 210 nm. The Inhibition potential of proteinase was determined using the below formula:

% Inhibition = {Absorbance of control/Absorbance of test} * 100

Preparation of RBCs suspension

5 mL of human blood was taken and added to 5 mL EDTA solution (7.5 g EDTA in 5 mL Millipore water) in a centrifuge tube. Centrifugation was carried out for 15 min at 1500 rpm centrifugation. The supernatant obtained was collected. RBC pellet was collected and suspended in cold distilled water and stored overnight at 4°C. Centrifugation was carried out using a Cooling Centrifuge at 4°C and 10,000 rpm for 15 min. The process was repeated, and the obtained pellet was mixed with 10 mL Tris-HCl buffer (1.21 g of Tris base in 8 mL distilled water and concentrated HCl–pH 7).[5]

Heat-induced hemolysis-HRBC membrane stabilization assay

The Control tube was loaded with 100 µL of distilled water and 100 µL of RBC suspension in an ELISA plate. 100 µL of plant extract solution was taken from various concentrations (100 µg, 200 µg, 400 µg, 600 µg, 800 µg, 1000 µg) and added to the

wells. 100 µL aspirin was taken as the standard drug out of the concentration prepared was 1000 µg/10 mL. The ELISA plate was incubated for 1 hr, and the OD values of the samples were recorded at 590 nm using a UV-vis spectrometer. Stability was calculated using the formula given below:

% Stabilization = {Absorbance of test sample/Absorbance of control} * 100

MTT assay

The cell viability was recorded employing the MTT reduction assay. In a 96-well plate, cells were planted and incubated until they reached an 80% confluency. The cells were subsequently incubated for 24 hr after being treated with an aqueous extract of *C. bonducella* (1 µg/mL). Each well received 10 µL MTT solution and was kept in the incubator for 4 hr. The insoluble crystals were dissolved by aspirating the medium and then adding DMSO, which acts as a solubilizing agent. UV-vis spectrophotometer was employed to record absorbance at 570 nm. The percentage of cell viability was estimated by the formula given below:

Cell viability (%) = (O.D. of test sample / O.D. of control sample) * 100

Griess nitrite assay

RAW 264.7 macrophages were cultured using DMEM along with 10% FBS and 5% antibiotic (100 X Liquid in 10,000 U Penicillin and 10mg Streptomycin per mL in 0.9% normal saline in a 5% CO2 environment). 1 g/mL LPS was stimulated to assess the inhibitory effect of *C. bonducella* aqueous extract. These cultured cells were loaded onto a 96-well plate and incubated till 80% confluency was achieved. These cells were then left for co-incubation for 24 hr with LPS (1 µg/mL) and water. The NO synthase inhibitor L-NAME (200 µM) was used as a positive control. The supernatant was used to estimate nitrite using the Griess nitrite test after incubation. The amount of the supernatant and Griess reagent containing 1% sulfanilamide and 0.1% naphthyl ethylene diamine in 5% phosphoric acid were stirred together and left for incubation at 37°C for 10 min. UV-vis spectrophotometer was used to record the absorbance at 540 nm.[6]

Phytochemical estimation

Qualitative analysis

Test for saponins

The plant extract was added to 20 mL Millipore water in a graduated cylinder and was shaken for 15-20 mins. The formation of a white foam layer of 0.5-1 cm indicates the presence of saponins.

Test for proteins

Biuret test–3 mL plant extract along with 3 mL 4% (0.4g in 10 mL) sodium hydroxide was added to a test tube. Subsequently, a few drops of 1% (0.1g in 10 mL) copper sulfate solution there added to get a purple solution.[7]

Test for carbohydrates

In a test tube, 2 mL plant extract was added along with 1 mL Molisch reagent. Subsequently, add drops of concentrated H_2SO_4. The formation of the purple ring detects positive results.[7]

Test for tannins

1 mL plant extract and 2 mL 5% (0.5 g in 10 mL) ferric chloride were added to a test tube. Positive results will be indicated by dark blue or greenish black colour.[7]

Test for phenols

1 mL plant extract and 2 mL 10% (1 g in 10 mL) lead acetate was added to a test tube. Brown ppt. indicates presence.[7]

Test for flavonoids

2 mL plant extract and 1 mL 2N NaOH (0.8 g in 10 mL) were mixed. The yellow colour solution indicates presence.[7]

Test for alkaloids

1 mL plant extract and 1 mL Wagner reagent (0.2 g of iodine in 0.6 g of potassium iodide in 10 mL) were mixed. Reddish brown precipitate indicates the presence of alkaloids.[7]

Quantitative analysis

Alkaloids

5g of seed powder was added to 200 mL of 10% acetic acid in a beaker. The made-up solution was covered and kept aside for 4 hr. The extract was maintained in a water bath until 1/4th of the original amount was obtained after filtering the solution using Whatman filter paper. Conc. Ammonium hydroxide was added dropwise till the precipitate was sedimented at the bottom of the beaker. The precipitate was again filtered and powdered using a lyophilizer. The sample was sent for GC-MS profile study.[8] The dried sample was weighed, and the percentage of total alkaloids present was determined using the below formula:

% alkaloids = {Weight of residue (g)/Weight of sample taken (g)} * 100

Flavonoids

To 2.5 g of sample, 50 mL of 80 per cent aqueous methanol was added and stored in a beaker and left covered for 24 hr

Table 1: Yield and % yield of different extracts.

Sl. No.	Extracts	Yield (grams)	Yield (%)
1	Hexane	8.05±0.35	16.1
2	Dichloromethane (DCM)	3.02±0.16	6.04
3	Ethyl Acetate (EA)	9.00±0.28	18
4	Methanol	11.00±0.42	22
5	Aqueous Solution	8.00±0.37	16

Table 2: Membrane stabilization (%) of different extracts.

Concentration	Hexane	DCM	EA	Methanol	Aqueous	Aspirin
100 µg	5±0.047	8±0.062	3±0.026	4±0.062	12±0.053	73±0.33
200 µg	6±0.055	10±0.07	6±0.048	8±0.037	33±0.26	84±0.33
400 µg	11±0.072	10±0.05	11±0.050	12±0.328	35±0.075	85±0.092
600 µg	23±0.095	16±0.42	24±0.051	15±0.039	47±0.10	86±0.23
800 µg	40±0.091	24±0.088	44±0.128	20±0.173	64±0.33	90±0.45
1000 µg	45±0.099	59±0.093	55±0.0033	23±0.393	75±0.096	92±0.05

undisturbed at room temperature. The supernatant was discarded, and the residue was extracted with 50mL of ethanol three times. The solution was kept in a water bath after filtering it using Whatman filter paper. The content was later lyophilized and weighed.[9] The percentage of flavonoids was determined using the below formula:

$$\% \text{ flavonoids} = \{\text{Weight of residue (g)}/\text{Weight of sample taken (g)}\} * 100$$

GC-MS profile study

1 mg alkaloid extract was dissolved in 1 mL aqueous methanol and passed through the GC-MS apparatus. The following were the experimental settings for capillary GC-MS analysis: Capillary column HP-5, the temperature of the detector was kept at 280°C, the temperature of injector at 250°C, carrier gas He (1 mL/min), split ratio 1/20, the volume of injection 0.2 L and mass range (m/z) 20–440.[10]

RESULTS

Extraction of plant material

The yield percentage for different extracts calculated has been shown in Table 1. The methanolic extract shows the maximum yield, then Ethyl acetate, then aqueous, and the least was shown by hexane extract. It was observed that DCM showed the most negligible yield percentage.

In vitro anti-inflammatory assays

Membrane Stabilization

The maximum stabilization observed was 75±0.096% of the aqueous extract at 1000 µg/mL, and the maximum inhibition of Aspirin was 92±0.05 at 1000 µg/mL (Table 2). The other test extracts also showed potency in stabilizing the RBC membrane. Hence, it is understood that aqueous extract shows the highest anti-inflammatory efficacy.

Albumin denaturation assay

The maximum inhibition observed was 125±0.25 of the aqueous extract at 1000 µg/mL, and the maximum inhibition of Aspirin was 142±0.58 at 1000 µg/mL (Table 3). Hence, it is understood that aqueous extract shows the highest anti-inflammatory efficacy.

Proteinase inhibition assay

The maximum inhibition observed was 106±0.15 of the aqueous extract at 1000 µg/mL, and the maximum inhibition of Aspirin was 138±0.052 at 1000 µg/mL (Table 4). Hence, it is understood that aqueous extract shows the highest anti-inflammatory efficacy.

MTT assay

MTT reduction assessed the cytotoxicity effect of aqueous extract of *C. bonducella* RAW rat 264.7 macrophages. The viability of cells in the presence of the aqueous extract is shown in Figure 2. The results showed that even at the most significant concentration (10 µg), the aqueous extract of *C. bonducella* had no effect on cell viability and was used in further research.

Methanol was used as a vehicle and 0.01% Triton X-100 was used as the positive control. Statistical evaluation was done by one-way ANOVA followed by Dunnett's Multiple Comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, $P < 0.05$ as compared with control.

Griess assay

In RAW 264.7 macrophages, the effect of the aqueous extract of *C. bonducella* on LPS-induced nitrite inhibition was evaluated (Figure 3). The released nitrite by the supernatant of the cultured

Table 3: Albumin denaturation inhibition (%) of different extracts.

Concentration	Hexane	DCM	EA	Methanol	Aqueous	Aspirin
100 µg	16±0.095	24±0.022	64±0.45	64±0.33	50±0.075	75±0.092
200 µg	19±0.56	27±0.24	68±0.064	64±0.084	66±0.067	80±0.56
400 µg	23±0.075	35±0.15	74±0.082	75±0.57	83±0.39	84±0.045
600 µg	23±0.23	38±0.088	76±0.56	76±0.34	87±0.23	88±0.34
800 µg	42±0.42	54±0.057	85±0.095	83±0.078	88±0.44	95±0.089
1000 µg	48±0.078	58±0.097	86±0.076	85±0.46	125±0.25	142±0.58

Table 4: Proteinase inhibition (%) of different extracts.

Concentration	Hexane	DCM	EA	Methanol	Aqueous	Aspirin
100 µg	24±0.34	33±0.59	65±0.33	70±0.075	51±0.45	66±0.075
200 µg	33±0.076	38±0.087	67±0.45	73±0.065	56±0.31	72±0.069
400 µg	36±0.24	47±0.45	72±0.081	79±0.097	85±0.082	87±0.082
600 µg	45±0.087	52±0.33	75±0.29	80±0.35	91±0.52	118±0.077
800 µg	46±0.16	58±0.17	82±0.59	85±0.067	97±0.098	127±0.13
1000 µg	46±0.077	62±0.068	85±0.082	93±0.093	106±0.15	138±0.052

Figure 1: Preparation of Plant Extract.

cells was recorded utilizing the Griess reagent. The cells which were not treated produced fewer amounts of nitrate (5.2 ± 0.016 µM), but LPS-treated cells produced higher levels of nitrite (27.1± 0.110 µM). The aqueous extract of *C. bonducella* showed a substantially dose-dependent inhibitory effect on nitrite generation (p<0.05). Compared to the LPS- treated cells, the positive control called L-NAME helped reduce nitrite generation at 200 µM (p<0.05).

The phytochemical tests showed alkaloids, phenols, proteins, carbohydrates and saponins in hexane extract. The DCM extract contained proteins, carbohydrates, and a trace amount of alkaloids and phenols. In ethyl acetate extract, alkaloids,

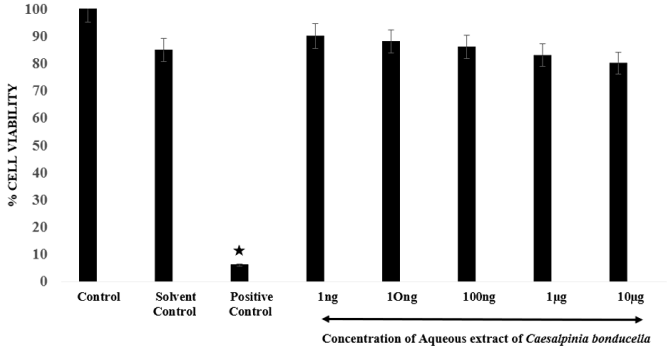

Figure 2: Effect of aqueous extract of *C. bonducella* on cell viability in RAW 264.7 macrophages employing MTT assay.

Table 5: Phytochemical constituents of various extracts of *C. bonducella* (Qualitative analysis)

Phytochemicals	Hexane	DCM	EA	Methanol	Aqueous
Alkaloids	+		+	++	++
Flavonoids	-	-	+	++	+
Phenols	+		+	+	-
Tannins	-	-	-	-	-
Proteins	+	+	+	+	-
Carbohydrates	+	++	+	++	
Saponins	+	+	+	+	-

Table 6: GC-MS analysis of alkaloids isolated from aqueous extract of *C. bonducella* GC-MS.

Sl. No	Name of compound	Molecular Formula	Molecular Weight	Retention time	% peak area
1	1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester	$C_{16}H_{22}O_4$	278	16.229	3.406
2	Diisobutyl phthalate	$C_{16}H_{22}O_4$	278	17.038	18.388
3	Phthalic acid, butyl 2-pentyl ester	$C_{18}H_{26}O_4$	292	17.329	5.875
4	Phthalic acid, butyl hex-3-yl ester	$C_{19}H_{26}O_4$	306	17.856	2.009
5	Phthalic acid, butyl hept-4-yl ester	$C_{19}H_{28}O_4$	320	18.514	3.518
6	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	25.331	4.659
7	1,2,4,5,6,6a,7,11b-Octahydro-6-hydroxy -9-methoxy-6,11b-dimethyl-3H benz[de]anthracen-3-one	$C_{20}H_{24}O_3$	312	29.986	2.815
8	Acetic acid, 17-(1-acetoxy-ethyl)-10,13-dimethyl-3-oxo-2,3,8,9,10,11,12,13, 14,15, 16,17-dodecahydro-1H -cyclopenta[a]phenanthren-11-yl (ester)	$C_{25}H_{36}O_5$	414	35.355	29.147

flavonoids, phenols, proteins, saponins, and carbohydrates were present. Alkaloids, phenols, flavonoids, proteins, carbohydrates, and saponins were present in methanolic extract. The aqueous extract's presence of alkaloids, flavonoids, and a trace amount of carbohydrates was present (Table 5).

Quantitative analysis

Alkaloids

The weight of the dry residue was found to be 551 mg. The percentage of total alkaloids present in the 5g sample was observed as 11.02%.

Flavonoids

The weight of the dry residue was found to be 301 mg. The percentage of total flavonoids in the 5g sample was 6.02%.

Statistics

Statistical evaluation was done by one-way ANOVA followed by Dunnett's Multiple Comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, $p < 0.05$ as compared with control

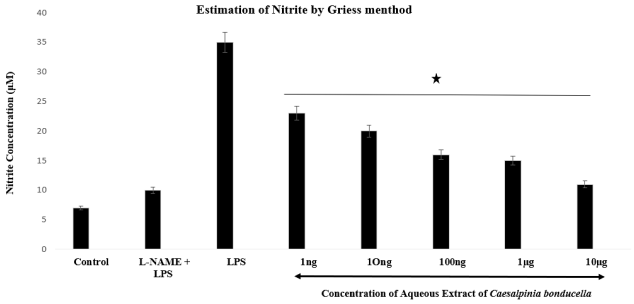

Figure 3: Effect of aqueous extract of *C. bonducella* on nitrite inhibition in LPS stimulated RAW 264.7 macrophages.
Statistical evaluation was done by one-way ANOVA followed by Dunnett's Multiple Comparison test and expressed as mean ± S.D in triplicates of three independent experiments. *, p < 0.05 as compared with LPS.

GC-MS

As alkaloids were present in higher amounts, it was sent for GC-MS profile study, and it was found that 8 peaks (compounds)

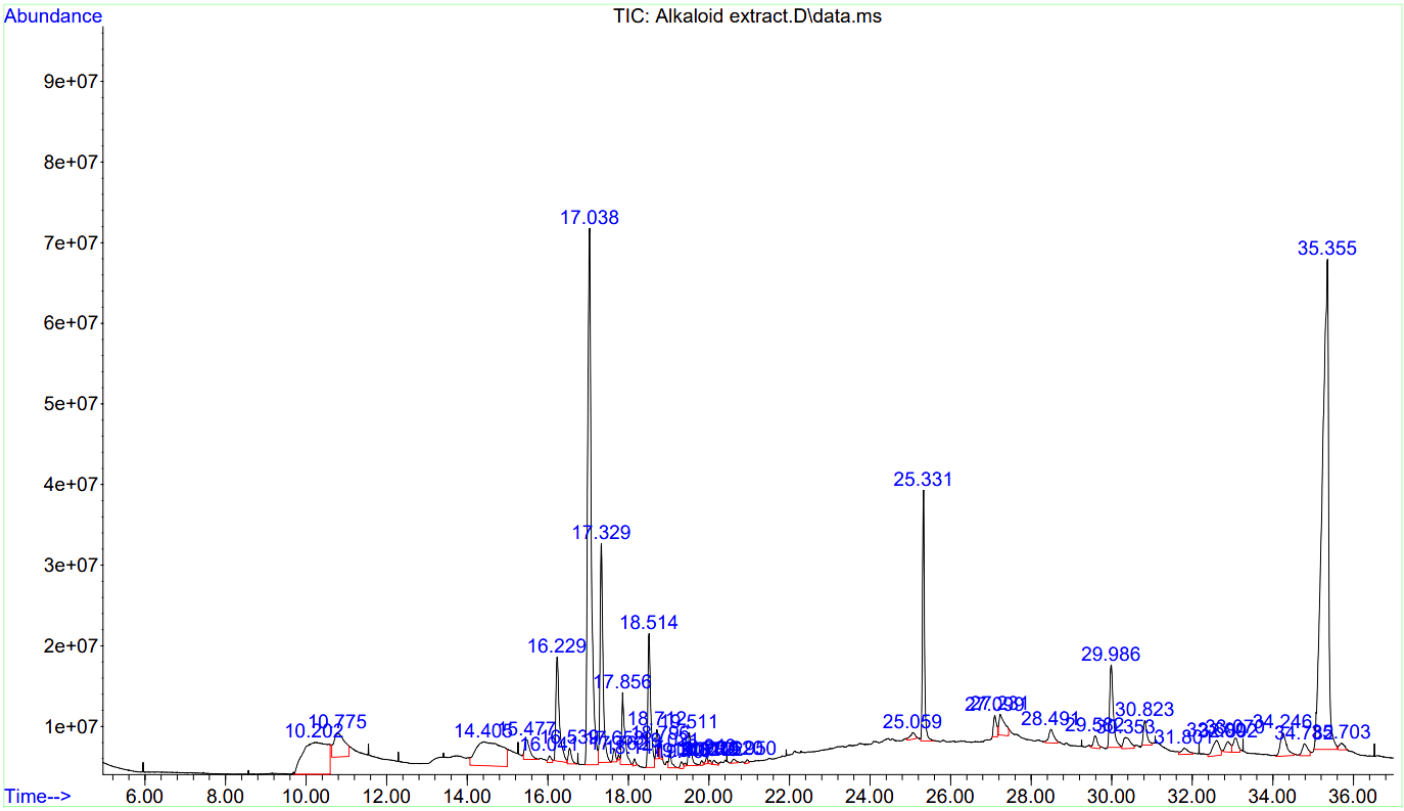

Figure 4: GC-MS profile study of alkaloids isolated from *C. bonducella* kernel powder.

were predominantly present (Table 6) which have medical applications (Figure 4).

DISCUSSION

Qualitative analysis

Various *in vitro* anti-inflammatory assays have been used in this evaluation. Firstly, HRBCs mimic the lysosomal membrane, so an assay for stabilising the membrane of human red blood cells is carried out which will further protect the membrane from degrading at the time of inflammation.[11] Since, neutrophils (phagocytic cells) have an abundant amount of serine proteinase and leukocyte proteinase, which mediates the development of tissue damage, the seed in study is investigated for anti-proteinase activity.[12] It is known that proteins lose their biological function during inflammation due to modification in their secondary/tertiary structure along with high amount of nitric oxide being released which increases the pH and subsequently leads to accumulation of toxins in the tissue's microenvironment.[13]

Lipopolysaccharide (LPS) plays a significant part in inducing inflammatory responses that cause various diseases and disorders. LPS is an extracellular component of gram-negative bacteria capable of interacting with specific receptors on host effector cells. This further acts as a mediator and leads to the expression of pro-inflammatory cytokines.[14] Immune cells such as the macrophagic cell line are capable of portraying as a suitable model for *in vitro* inflammatory cells that perform pinocytosis and release nitric oxide (NO). The same cells are also capable of killing target cells via antibody-dependent cytotoxicity.[15] Furthermore, MTT assay is a cell viability assay that measures the changes taking place morphologically or in membrane permeability by excluding or taking up particular dyes since the sample, which is cytotoxic, would inhibit the proliferation of cells, thereby killing them.[16]

GC-MS works on the principle that components of the sample get separated on heating, and the vapours of the volatile compound are carried through an inert gas column. This opens and passes the components into the MS column where they get separated based on the mass of the analyte molecule.[17]

CONCLUSION

C. bonducella is an Indian herb that has great potential to cure many diseases. Since this plant is widely available, it is easier to obtain in large quantities at a cheaper cost. Hence, we focused on the seeds by extracting the bioactive leads responsible for curing anti-inflammatory diseases that can help advance herbal drugs. The current study reveals that an aqueous extract of *C. bonducella* kernel powder possesses anti-inflammatory properties.

Alkaloids, phenols, flavonoids, proteins, saponins, and carbohydrates were screened using different phytochemical tests in methanolic extract. Hexane extract showed presence of alkaloids, phenols, proteins, carbohydrates, and saponins, whereas ethyl acetate showed positive result for alkaloids,

proteins, phenols, saponins, flavonoids, carbohydrates, and tannins. In DCM extract, the presence of proteins, carbohydrates, and a trace amount of alkaloids and phenols was observed. Lastly, in the aqueous extract, alkaloids, flavonoids, and a trace amount of carbohydrates were found, although alkaloids have been given preference due to its higher concentration and better efficacy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

***C. bonducella*:** *Caesalpinia bonducella*; **PCOS:** Polycystic Ovary Syndrome; **DCM:** Dichloromethane; **EA:** Ethyl Acetate; **HRBC:** Human Red Blood Cell; **GC-MS:** Gas Chromatography–Mass Spectrometry; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **LPS:** Lipopolysaccharide; **NF-Kb:** Nuclear factor kappa B; **JAK/STAT:** Janus kinase/Signal transducers and activators of transcription; **MAPK:** Mitogen-activated protein kinase; **ICAM:** Intercellular adhesion molecule; **IL:** Interleukin; **TNF:** Tumor necrosis factor; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **HIV:** Human immunodeficiency virus; **BSA:** Bovine serum albumin; **ELISA:** Enzyme-linked immunosorbent assay; **L-NAME:** L-NG-nitroarginine methyl ester.

SUMMARY

Inflammatory diseases have become one of the significant causes of concern since they require immediate medical attention. Approximately 6.8 million people in the world are affected by inflammatory diseases. Patients experience various adverse effects from non-steroidal anti-inflammatory drugs (NSAIDs) and steroidal drugs. Hence, herbal medicines are becoming the prime focus area for researchers and medical practitioners. In this study, seven phytochemical tests of *C. bonducella* were conducted post which, alkaloids (quinolizidine) were observed to be highest in proportion apart from carbohydrates and flavonoids. The attenuation of pro-inflammatory mediators were highly evidenced in the Aqueous extract which further needed unravelling of the constituents in the Alkaloid fraction. The GC-MS profiling of the alkaloid showed predominantly 8 peaks/compounds present. This needs to further subjected to preparative HPLC and test for their individual efficacy.

REFERENCES

1. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, *et al*. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2018;9(6):7204-18. doi: 10.18632/oncotarget.23208, PMID 29467962.
2. Dharmadeva S, Galgamuwa LS, Prasadinie C, Kumarasinghe N. *In vitro* anti-inflammatory activity of *Ficus racemosa* L. bark using albumin denaturation method. Ayu. 2018;39(4):239-42. doi: 10.4103/ayu.AYU_27_18, PMID 31367147.
3. Beaudeux JL, Giral P, Bruckert E, Foglietti MJ, Chapman MJ. Matrix metalloproteinases, inflammation and atherosclerosis: Therapeutic perspectives. Clin Chem Lab Med. 2004;42(2):121-31. doi: 10.1515/CCLM.2004.024, PMID 15061349.
4. Reddy A, Sundaresan S. Phytochemical analysis and suppression of inflammatory targets by *Adathoda vasica*. Asian J Pharm Clin Res. 2018;11(5):162-6. doi: 10.22159/ajpcr.2018.v11i5.24243.
5. Chowdhury A, Azam S, Jainul MA, Faruq KO, Islam A. Antibacterial activities and *in vitro* anti-inflammatory (membrane stability) properties of methanolic extracts of *Gardenia coronaria* leaves. Int J Microbiol. 2014;2014:410935. doi: 10.1155/2014/410935, PMID 24695677.
6. Amala R, Sujatha S. Presence of pyrroloquinazoline alkaloid in *Adhatoda vasica* attenuates inflammatory response through the downregulation of pro-inflammatory mediators in LPS stimulated RAW 264.7 macrophages. Bio Impacts. 2021;11(1):15-22. doi: 10.34172/bi.2021.03, PMID 33469504.
7. Sorescu A, Nuta A, Iancu RI. Qualitative analysis of phytochemicals from sea buckthorn and gooseberry. Intech Open; 2018.
8. Harborne JB. Phytochemical methods: A guide to modern techniques of plant analysis. London: Chapman and Hall Ltd.; 1973. p. 279.
9. Ejikeme C, Ezeonu CS, Eboatu AN. Determination of Physical and Phytochemical Constituents of some Tropical Timbers Indigenous to nigerdelta area of Nigeria. Eur Sci J. 2014;10(18):247-70.
10. Erdemoglu N, Ozkan S, Duran A, Tosun F. GC-MS analysis and antimicrobial activity of alkaloid extract from *Genista vuralii*. Pharm Biol. 2009;47(1):81-5. doi: 10.1080/13880200802448674.
11. Anosike CA, Obidoa O, Ezeanyika LU. Membrane stabilization as a mechanism of the anti-inflammatory activity of methanol extract of garden egg (*Solanum aethiopicum*). Daru. 2012;20(1):76. doi: 10.1186/2008-2231-20-76. PMID 23351977.
12. Manikandas S, Vadivel V, Brindha P. Studies on Nutraceutical Properties of *Caesalpinia bonducella* L.: An Indian Traditional Medicinal Plant. Res J Med Plants. 2016;10(2):127-39. doi: 10.3923/rjmp.2016.127.139.
13. Gutiérrez AJ, Rubio C, Caballero JM, Hardisson A. Academic press. Nitrites, Encyclopedia of Toxicology. 3rd ed. 2014:532-5.
14. Cao Y, Chen J, Ren G, Zhang Y, Tan X, Yang L. Punicalagin prevents inflammation in LPS-induced RAW264.7 macrophages by inhibiting FoxO3a/Autophagy signaling pathway. Nutrients. 2019;11(11):2794. doi: 10.3390/nu11112794, PMID 31731808.
15. Taciak B, Białasek M, Braniewska A, Sas Z, Sawicka P, Kiraga Ł, *et al*. Evaluation of phenotypic and functional stability of RAW 264.7 cell line through serial passages. PLOS ONE. 2018;13(6):e0198943. doi: 10.1371/journal.pone.0198943, PMID 29889899.
16. Ghasemi M, Turnbull T, Sebastian S, Kempson I. The MTT assay: Utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. Int J Mol Sci. 2021;22(23):12827. doi: 10.3390/ijms222312827, PMID 34884632.
17. Kumar MH, Rao PK, Sundaram MRK, Shil RL. S, Kumar A. The GC MS study of one Ayurvedic medicine, Vasakadyaristam. Alcohol. 2019;8(122.1):36666635.

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