AN *IN-VITRO* STUDY ON ANTI-BACTERIAL AND ANTI-INFLAMMATORY AND ACTIVITIES OF ACETONE EXTRACT FROM THE LEAVES OF *CADABA FRUTICOSA*

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Abstract

The present study was to evaluate the antibacterial and anti-inflammatory, phytochemical composition of acetone extracts from the leaves of *Cadaba fruticosa*. Phytochemicals were analyzed through chemical tests, thin layer chromatography (TLC). Acetone extract of *Cadaba fruticosa* were evaluated for antibacterial potential via the agar well diffusion method against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Klebsiella pneumonia*. The minimal inhibitory concentration (MIC) were determined by the microdilution method. *In vitro* anti-inflammatory activity of the acetone extract of *Cadaba fruticosa* was evaluated using albumin denaturation, inhibition of lipoxygenase and proteinase inhibitory assays. Acetone extract of *Cadaba fruticosa* had the highest phenolic and flavonoid contents (60.12±1.78 µg GAE/g, 54.78±2.41 µg QE/g. Acetone extract considerably inhibited the growth of the bacteria *S. aureus, E. coli* and *K. pneumonia*. *In vitro* anti-inflammatory potential at a concentration of 100 µg/mL of acetone extract of *Cadaba fruticosa*. The lowest EC_{50} (60.89 and 56.78 µg/mL) observed in the albumin denaturation and inhibition of proteinase assay. Positive correlations were observed between total phenolics, antibacterial and anti-inflammatory potential of the selected plant extracts, indicating a significant contribution of phenolic compounds in the plant extracts to these activities.

1. INTRODUCTION

To offset the lack of new antibacterial and the increase of antibiotic struggle, herbals could exemplify a possible solution. Definitely, plants are armed with an display of effective defense mechanisms, such as the construction of secondary metabolites, to fight pests and pathogens before they are able to cause serious damage. Plants and microbes organisms have co-evolved for more

than 350 million years and have industrialized approaches to incredulous each other's defense systems. Plant non nutrients play a main role in what way plants acclimate to their environment; they also act as their surveillance system. They are byproducts of odors, tastes and colors of plant tissues are responsible for the specific. Plant secondary metabolites can also help the plant to cope with abiotic stresses (e.g., UV radiation) and to communicate with other organisms (e.g., herbivores, pathogens, neighboring plants, pollinators and fruit dispersers), so they are also important for growth and development. These compounds usually belong to one of three large chemical classes known for biological activity: terpenoids, phenolics and alkaloids.

Old-style remedial handling in India, Siddha and Ayurveda medicines are used numerous plants in the form of chooranam, decoction, syrup, leghyam and tablets. These preparations from sashthiya or property medicine addressed a number of therapeutic complications in reproducible method, quickly or at times slowly, apparently due to alterations in their amount of bioactive chemicals. These remarks by means of some plant preparations for numerous disorders incited us to appearance into their attention of bioactive molecules or develop indicator examines which could authenticate consistency of their presentation (Ben Salem *et al.,* 2015). Different therapeutic plants have been evaluated for their capability to anti-microbial and inflammatory related diseases (Chen *et al.,* 2016). Old-style therapeutic herbals are frequently economically favorable, easily available, and simply consumable substitutes for marketable medicines. Roughly 60-80% of the all over the country population still depend on on old-style remedies for the handling of collective infections.

Above the previous eras, exhaustive efforts have been made to determine herbal plants retaining bacterial infection for control numerous diseases (Atanasov *et al.,* 2015). The phytomedicines are attractive additional scientifically constructed, with aggregate importance positioned on confirmed product protection and efficiency (Asmelashe *et al.,* 2017). Medicinal plants contained rich sources nonnutritive molecules as antimicrobial agents. A varied assortment of therapeutic plants parts are recorded to get variability of phyto-molecules which hold altered remedial properties against microbes and physiological behavioral diseases. Even though so many plants species have been confirmed for against microbes, the common of these have not been sufficiently assessed.

Bacterial contaminations are one of the foremost reasons of infectious diseases in India. Deficiency and deprived health setup remain to be an impairment to operative well-being care facility distribution (Cos *et al.,* 2006). On a worldwide scale, serious infections caused by these pathogenic prokaryotic bacteria have develop a significant cause of illness and impermanence in immune-compromised patients in developing countries (Muhuha *et al.,* 2018). Even though the

convenience of a extensive range of antibiotics, bacteria are continuously emerging confrontation to these agents, which kinds it problematic for the determined effort of fighting infectious diseases. From the beginning of antimicrobial claim in treatment of bacterial diseases, bacteria responded by establishing diverse forms of mechanisms of resistance.

Inflammations are very clear may be as a worsening progression which is strong adequate to cause limited accretion of low molecular weight catabolic products, which in chance raises tissue osmotic pressure that interests extra fluid, with or without heat release adequate for important raise of tissue temperature. This progression is in a high-pitched distinction to the pathogenesis of burns, where superficially functional heat causes a method that is in essence conflicting to inflammation, comportment solitary some insincere resemblances with the latter. The inflammatory development is itself a pathological process, while the usual anti-inflammatory rejoinder that follows after acute inflammation have a habit of to converse tissue homeostasis towards ordinariness and must consequently be stared as a true defensive reaction of the unnatural tissue (Chtourou *et al.,* 2015).

Cadaba fruticosa is small shrub, leaves are simple, oblong, slightly pubescent; few flowered arranged racemes, greenish-white; sepals are four, obovate; petals are four, clawed; stamens are 6-8, exerted; ovary two celled, many ovule, stigma sessile, clavate, usually gynophore occur; Fruits 4 x 0.5 cm, siliqua unripe green in color when ripe fruit red in color; seeds globose. *Cadaba fruticosa* leaf extract are used in helminthiasis, uterine complaints, syphilis, antiphlogistic, fever, general weakness. Leaves also used in snake bite, antidotes for poisoning insects. Root decoction is recommended for round worm. Root bark is used in boils blister and cuts. The plant also effective for antidiabetic. Paste prepared from fresh leaves with milk used for neurological disease, advices for 3 days. *Cadaba fruticosa* leaf contained some alkaloids such as cadabin, cadabicine, stachidrine, 3-Hydroxy Stachydrine, Cadabalone. Aqueous extract of plant contains terpenoids, flavones, anthraquinones and sugars (Karthiyayini *et al.,* 2017).

2. MATERIALS AND METHODS

2.1. Plant collection and preparation of extracts

Cadaba fruticosa leaves was obtained from Herbal garden of Government Siddha Medical College, Arumbakkam, Chennai, Tamilnadu, India. A plant taxonomist authenticated the plant and samples were kept in the Medicinal Botany herbarium with voucher specimen numbers MB/GSMC-245/2021. *Cadaba fruticosa* leaves were washed thoroughly with distilled water and shade-dried for 3 d at room temperature. The dried leaves were uniformly ground using an electric grinder. The powdered plant material (250 g) was extracted for 4 d in 1 L 100% acetone

(Harborne, 1973). The separated extracts were then filtered through Whatman No. 1 filter paper and the acetone filtrate evaporated to dryness using a rotary evaporator at room temperature (30 °C). The thick extracted mass was then dried at room temperature, and the dried extract stored in an air-tight container at 4 °C until further use.

2.2. Total phenolic content

Total phenolic content was analyzed using the Folin–Ciocalteu colorimetric method (Chlopicka et al., 2012). An aliquot of 0.3 mL of the acetone extract of *Cadaba fruticosa* was mixed with Folin-Ciocalteu phenol reagent (2.25 mL). After 5 min, 6% sodium carbonate (2.25 mL) was added and the mixture was allowed to stand at room temperature for 90 min. The absorbance of the mixture was measured at 725 nm in a spectrophotometer. A calibration curve for gallic acid in the range 20-80 µg/mL was prepared in the same manner. Results were expressed as mg gallic acid equivalent (GAE) per gram extract.

2.3. Total flavonoid content

Total flavonoid content was determined using the aluminum chloride colorimetric method (Chang et al., 2002). A calibration curve for quercetin in the range 20–80 µg/mL was prepared. Acetone extract of *Cadaba fruticosa* (0.5 mL) and standard (0.5 mL) were placed in separate test tubes and 10% aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), 80% methanol (1.5 mL) and distilled water (2.8 mL) added and mixed. A blank was prepared in the same manner but 0.5 mL of distilled water was used instead of the sample or standard. All tubes were incubated at room temperature for 30 min and the absorbance was read at 415 nm. The concentration of flavonoid was expressed as mg quercetin equivalent (QE) per gram extract. Each plant extract was made in triplicate.

2.4. Thin layer chromatography

The acetone extract of *Cadaba fruticosa* (10 µL) were applied on pre-coated TLC plates using capillary tubes and air dried. The TLC plates were developed in a chamber using chloroform: methanol (5:1) as the mobile phase and observed under UV light (254 nm).

2.5. Assay for antibacterial activity

Antibacterial activity of the acetone extract of *Cadaba fruticosa* was resolute using the agar well diffusion method (Valgas et al., 2007). Newly prepared bacterial inoculum was consistently spread using a sterile cotton swab on the entire agar surface. The sterile disc (6 mm) was place on solidified media and different concentration of crude extract was poured into the disc. Petri plates were then allowed to stand at room temperature for 1 h and incubated at 37 °C overnight. Controls

were run in parallel whereby solvent was used to fill the sterile disc. The plates were observed for zones of inhibition after 24 h and the results compared with those of the positive control, streptomycin (10 µg/mL).

2.6. Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The determination of MIC of the acetone extract of *Cadaba fruticosa* was carried out by the microdilution method using nutrient broth. *C. fruticosa* extracts were dissolved in 10% DMSO and two-fold dilutions were prepared with culture broth. Each test sample contained different concentration of extracts and inoculated with 10 µL of bacterial suspension containing 5×10^6 CFU/mL and incubated for 24 h at 37 °C. Bacterial growth was detected by reading absorbance at 500 nm. Bacterial growth was indicated by a color change from purple to pink or colorless (assessed visually). MIC was defined as the lowest plant extract concentration at which the color changed, or the highest dilution that completely inhibited bacterial growth. Experiments were carried out in triplicate to test each dilution for each bacterial strain to determine MIC values.

2.7. Inhibition of albumin denaturation activity

The anti-inflammatory activity of acetone extract of *Cadaba fruticosa* was deliberate by inhibition of albumin denaturation was studied. The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adapted using few drops of 1 N HCl. The different concentration of acetone extract were incubated at 37°C for 20 min and then heated to 51°C for 20 min, successively chilled the test sample was measured at 660 nm. The experiment was repeated in triplicate (Montefusco *et al.,* 2013). The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition= (Abs Control−Abs Sample) 100/Abs control.

2.8. Heat-Induced Hemolysis

The reaction mixture (2 ml) consisted of 1 ml acetone extract of *Cadaba fruticosa* of different concentrations (25-100 µg/ml) and 1 ml of 10% red blood cells (RBCs) suspension, in its place of the test sample, the only saline was added to the control test tube. Diclofenac sodium was used as a standard drug. The centrifuge tubes containing reaction mixture were incubated in a water bath at 56°C for 30 min (Sadique *et al.,* 1989). The reaction mixture was again centrifuge for 5 min, the absorbance of the supernatants was taken at 560 nm. The experiment was performed in

triplicates for all the test samples. The percentage inhibition of hemolysis was calculated as follows:

Percentage inhibition = (Abs Control−Abs sample) X 100/Abs control.

2.9. Inhibition of Lipoxygenase Activity

5-LOX inhibition assay was performed using the principle of 1-4 diene (linoleic acid) oxidations to 1-3-diene. Briefly, an aliquot of the stock solution (50 µL, in dimethyl sulfoxide (DMSO) and tween 20 mixture; 29:1, w/w) of different concentration of acetone extract of *Cadaba fruticosa* was placed in a 3 mL cuvette, followed by addition of pre-warmed 0.1 M potassium phosphate buffer (2.95 mL, pH 6.3) and linoleic acid solution (48 µL). Thereafter, ice-cold buffer (potassium phosphate; 12 µL) was added with 5-LOX (100 U) and absorbance recorded at 234 nm (Baylac and Racine, 2003). The control was prepared with DMSO: tween 20 mixture (no enzyme inhibition).

2.10. Statistical analyses

Statistical evaluation was carried out by the SPSS software (SPSS Inc, Chicago, USA, ver. 13.0). Descriptive statistics were ascertained for all the contemplated attributes. Analyses were carried out in triplicate and the means of all parameters were examined for significance ($p < 0.05$) by analysis of variance (ANOVA).

3. RESULTS AND DISCUSSION

3.1. PHYTOCHEMICALS PROPERTIES OF *CADABA FRUTICOSA*

In this study, phytochemical screening of the acetone extract of *Cadaba fruticosa* was done to assess the availability of bioactive secondary metabolities. The presence of phytochemicals such as, flavonoids, alkaloids, tannins, steroids, phenols, saponins and terpenoids were detected. A previous study of Capparidaceae plants contain alkaloids, specifically spermidine, capparidisine, capparisine, capprisinine and N-acetyl capparisine (Shoib, 1986; Ahmed et al., 1992)

Table-1. Phytochemical screening of acetone extract of *Cadaba fruticosa*

Sl. No.	Phytochemical Constituents	Observation	Acetone extract of *Cadaba fruticosa*
1	**Alkaloids** -Dragendorff's Test -Mayers test	Orange / red precipitate Yellow or white precipitate	+ +
2.	**Flavonoids** -Alkalai Reagent -Lead acetate test	 Intense yellow colour Precipitate formed	 + +
3.	**Glycosides** Keller-Killiani test	Reddish brown colour ring formed	-
4.	**Tannin** -$FeCl_3$ test	Blue black coloration	-
5.	**Saponins** -Frothing test	Foam	+
6.	**Terpenoids** *-Salkowski test*	Dark reddish brown color in interface	-
7.	**Polyphenols** -Ferrozine test	Raddish blue	+
8.	**Anthocyanin test Ammonia**	Ammonia layer yellow in color	+

+ indicate positive result; -- Indicate negative result

3.2. TOTAL PHENOL AND FLAVONOID CONTENT

The examination presented that total phenolics (60.12±1.78 µg GAE/g and 58.78±2.41 µg Rutin/g plant extracts). Total flavonoid contents in the ethyl acetate extract of *Cadaba fruticosa* were calculated as rutin equivalent (µg/g) using the equation based on the calibration curve: y = 0.0182x - 0.0222, R2 = 0.9962, where y was the absorbance and x was the rutin equivalent (mg/g). Wang et al. (1989) reported that the greater the presence of phenolic hydroxyl groups higher antibacterial activity. Phenolic compound was causing protein denaturation of microbes through the pause of the enzymes action of metabolic reactions and cause the death of the micro-organism (Hasanudin et al., 2012). Flavonoids disrupt the integrity of the cell membrane in away they denature bacterial proteins and bacterial cell membrane damage (Hendi et al., 2011).

3.3. TLC PROFILE

TLC analysis of ethyl acetate extract of *Cadaba fruticosa* tested (Fig-1). In addition to the components with antimicrobial activity several compounds on the reference chromatogram were visible in UV light at 235 nm many of these compounds did not coincide with the antimicrobial

components. This could be qualified to evaporation of the active mechanisms, photooxidation or inadequate quantity of the active component (Masoko et al., 2005).

3.4. ANTIBACTERIAL ACTIVITY OF ACETONE EXTRACT OF *CADABA FRUTICOSA*

Antibacterial activity of acetone extract of *Cadaba fruticosa* tested against *Enterococcus faecalis, Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* were assessed as inhibition zones in the agar plates (Table-2). In this experimental all the bacteria were found to be sensitive to the polyphenol rich fraction. Additionally, the zone of inhibition reconsideration that the polyphenol rich fraction influenced antibacterial activity in proportion to concentration gradient ranges 25-100 µl/ml against the tested bacteria. Amongst the bacteria considered, *Staphylococcus aureus* and *Escherichia coli* was identified to be highly susceptible followed by *Pseudomonas aeruginosa* and *Enterococcus faecalis*. This may confirm the antibacterial property of acetone extract of *Cadaba fruticosa*. The antimicrobial potential of plant extracts can be attributed to the presence of certain bioactive compounds such as phenolics, alkaloids, flavonoids and terpenoids. Among all these biologically active compounds, Balouiri et al. (2016) confirmed alkaloids and phenolic acids as the most important and bioactive compounds against bacteria. Similarly, the results of antibacterial activities obtained in the present study were correlated to their total phenolic contents.

Table-2. The antibacterial activity of the acetone extract of *Cadaba fruticosa* by disc diffusion method

Pathogenic organism	Different concentrations acetone extract (µl/ml)			
	25 µl/ml	50 µl/ml	75 µl/ml	100 µl/ ml
Staphylococcus aureus	8.4±1.4	10.3±2.4	12.4±1.5	15.3±2.1
Pseudomonas aeruginosa	7.2±0.4	9.4±0.7	11.4±0.7	14.3±0.8
Escherichia coli	8.1±0.8	9.1±0.3	11.8±0.6	14.8±0.3
Enterococcus faecalis	7.5±1.6	8.7±1.8	10.3±2.1	13.8±1.8

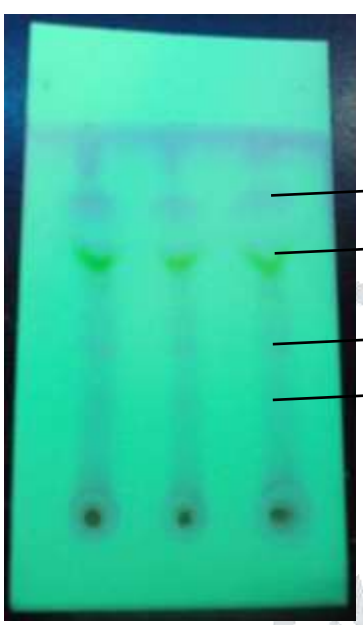
*The inhibitory Zone size measured included the 6.0 mm size of the well by means of caliper. All the assays were duplicated, and the mean values were recorded.

3.5. MINIMUM INHIBITORY CONCENTRATION

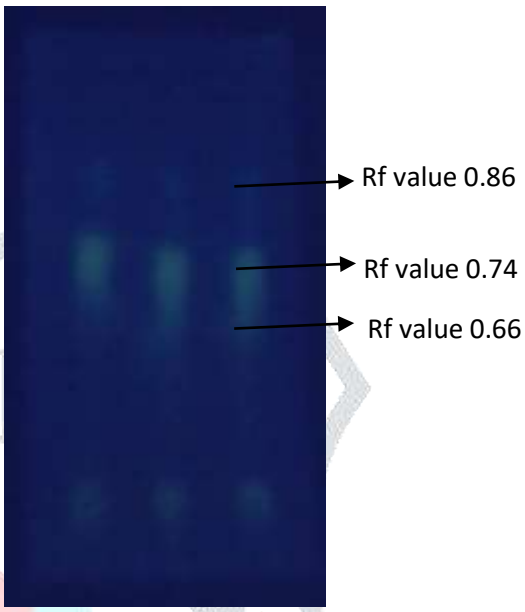
In the complete sequences, the MIC of ethyl acetate extract of *Cadaba fruticosa* ranged between 25 to 100 µg/ml against gram positive bacteria and gram negative bacteria, (*Staphylococcus aureus, Enterococcus faecalis, Escherichia coli* and *Pseudomonas aeruginosa*) respectively. The Minimum inhibitory absorption value of acetone extract of *Cadaba fruticosa* increases with increase in concentration. *S. aureus* exhibited maximum inhibition when compared

to the other pathogenic bacteria at 100 µl/ml concentration. *Enterococcus faecalis, Escherichia coli* appearances reasonable range of inhibition activity. *P. aeruginosa* display slighter activity. In comparison with gram positive bacteria and gram negative bacteria, the MIC of ethyl acetate extract of *Cadaba fruticosa* displayed highest inhibition in gram negative bacteria and among the gram positive bactertia *S. aureus* showed maximum inhibition (Fig-2).

Fig-1. TLC analysis of acetone extract of *Cadaba fruticosa*

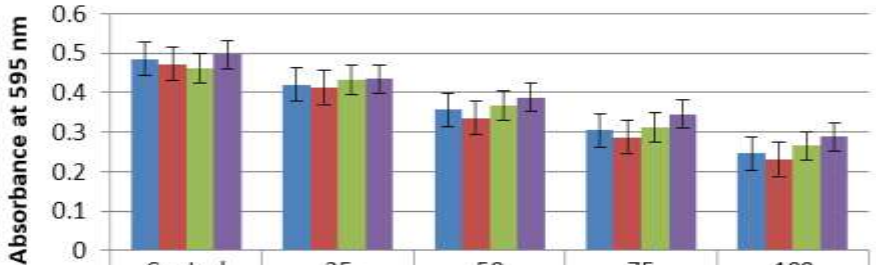


TLC plate Viewed UV Light at 240 nm



TLC plate Viewed UV Light at 360 nm

Fig-2. MIC OF ACETONE EXTRACT OF CADABA FRUTICOSA



	Control	25	50	75	100
Staphylococcus aureus	0.486	0.421	0.356	0.305	0.246
Escherichia coli	0.473	0.412	0.336	0.287	0.231
Enterococcus faecalis	0.462	0.433	0.368	0.312	0.265
Pseudomonas aeruginosa	0.497	0.435	0.387	0.345	0.289

3.6. PROTEIN DENATURATION INHIBITION OF ACETONE EXTRACT OF *CADABA FRUTICOSA*

Examination of acetone extract of *Cadaba fruticosa* of momentous activity on inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that alkaloid extract is proficient of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease. The maximum percentage inhibition of protein denaturation was observed as 73.21% at 100µg/ml which was close to the percentage of inhibition of diclofenac sodium 67.21%) (Table-3).

Table-3. Inhibition activity of protein denaturation of acetone of *Cadaba fruticosa*

Different concentration of extract	Inhibition percentage of protein denaturation	Diclofenac sodium (+ve control)
25 µl/ml	18.32±1.78	16.31±0.23
50 µl/ml	31.24±2.31	28.34±1.78
75 µl/ml	48.32±1.28	44.31±0.23
100 µl/ml	73.21±0.28	67.21±2.45
EC_{50} Value	74.34	81.23

Results are expressed as percentage inhibited inhibition of protein denaturation with respect to control. Each value represents the mean+SD of five experiments

3.7. HEAT-INDUCED HEMOLYSIS

The acetone extract of *Cadaba fruticosa* was considered for *invitro* anti-inflammatory activity. The acetone extract of *Cadaba fruticosa* showed maximum heat induced hemolysis 76.31% at a maximum concentration of 100 µg/mL in hypotonic solution. Furthermore, results were compared with the standard diclofenac, which showed (Table-4). The plant compounds exhibited membrane stabilization by inhibiting hypotonicity-induced lyses of the erythrocyte membrane (Yang et al., 2010), and its stabilization implies that the extract may stabilize lysosomal membranes. Plant metabolites such as flavonoids and tannin are known to be effective in reducing acute inflammation. The anti-inflammatory activity of the extract/fraction may be due to the presences of flavanoids, tannins etc. either singly or in combination (Sudharshan et al., 2010). In vitro result suggests that the acetone extract of *Cadaba fruticosa* possess potential anti-inflammatory activity.

Table-4. Heat induced hemolysis activity of acetone extract of *Cadaba fruticosa*

Different concentration of extract	Ethyl acetate extract of *Cadaba fruticosa*	Standard Diclofenac sodium
25 µl/ml	21.23±1.46	18.34±1.78
50 µl/ml	39.64±1.67	34.21±2.34
75 µl/ml	57.31±2.89	82.31±1.89
100 µl/ml	76.31±1.45	71.32±2.16
EC_{50} Vlaue	77.31±2.14	81.37±1.47

Results are expressed as percentage inhibited Lipoxygenase with respect to control. Each value represents the mean+SD of five experiments.

3.8. LIPOXYGENASE INHIBITION ACTIVITY OF ACETONE EXTRACT *CADABA FRUTICOSA*

The inhibition of Lipoxygenase using linoleic acid as substrate was determined for the anti-inflammatory activity in the acetone extract of *Cadaba fruticosa.* The acetone extract of *Cadaba fruticosa* at 100µl/ml concentration exhibited more inhibition than the other concentration. The inhibition percentage was above 69.31% at 100µl/ml (Table-5). The standard diclofenac sodium was showed 66.34% inhibition at 100 µg/mL. The acetone extract of *Cadaba fruticosa* was showed higher inhibition activity than positive control. Lipoxygenase catalyzes the addition of molecular oxygen to fatty acids containing a *cis*, *cis*-1, 4-pentadiene system. This reaction originates unsaturated fatty acid hydroperoxides. These products are further converted into others that play a key role in inflammatory processes. Hence, compounds which are able to inhibit that enzyme can be considered as antioxidants and possessing anti-inflammatory properties (Akinwunmi and Oyedapo, 2015).

Table-5. Inhibition activity of Lipoxygenase of acetone extract extract of *Cadaba fruticosa*

Different concentration of extract	Acetone extract of *Cadaba fruticosa*	Standard Diclofenac sodium
25 µl/ml	19.32±0.78	16.34±2.14
50 µl/ml	35.24±0.58	31.25±1.63
75 µl/ml	53.34±0.23	46.34±2.89
100 µl/ml	69.31±2.45	66.34±1.78
EC_{50} Vlaue	84.56±0.89	89.32±2.52

Results are expressed as percentage inhibited Lipoxygenase with respect to control. Each value represents the mean+SD of five experiments.

CONCLUSION

In conclusion, results specify that the acetone extract of *Cadaba fruticosa* possess antibacterial and anti-inflammatory properties at varying levels. Leaves of *Cadaba fruticosa* showed higher antibacterial, denaturation of protein and lipoxygenase inhibition activity. Pearson's correlation studies showed that there were significant correlations between estimated antibacterial and anti-inflammatory properties. Results indicate that these antibacterial and anti-inflammatory activities may be due to the occurrence of bioactive phenolic compounds in these leaf of *Cadaba fruticosa*.

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