

# Studies on *in-vitro* Anti-inflammatory activity of *Acmella oleracea* metabolic compounds

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## ABSTRACT

The present investigation exposed that the extracts of *Acmella Oleracea* plant have potent phytochemical and antimicrobial activity which explains its use in traditional system of medicines. The qualitative analysis of the extracts from the leaf sample of *Acmella Oleracea* showed the presence of phytochemical constituents such as tannins, saponin, flavonoids, steroid, lipids, amino acids and terpenoids. The extracts of *Acmella Oleracea* were originated to be more or less active against almost all tested pathogenic strains. Hence, *Acmella Oleracea* can source of natural antimicrobials that can serve as a substitute to conventional medicines. The presence of Anti-inflammatory agent in *Acmella Oleracea*. *Acmella Oleracea* can be considered as a resource for potential anti-inflammatory and antimicrobial agents.

**Key words:** *Acmella Oleracea*, terpenoids, and Anti-inflammatory.

## INTRODUCTION

Inflammation can be classified as either *acute* or *chronic*. *Acute inflammation* is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as *chronic inflammation*, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process.

The inflammatory process is a combination of many pathways like a synthesis of prostaglandin, interleukin or other chemo toxin, adhesive protein receptor action, platelet-activating factors. All can act as chemotactic agonists. Inflammation initiates with any stress on the membrane or by other trigger or stimuli, these activate hydrolysis of membrane phospholipid by phospholipase - A into arachidonic acid, which further substrate for cyclo oxygenase and lipo oxygenase enzyme and byproduct of these are prostaglandins PGE<sub>2</sub>, PGH<sub>2</sub> and leukotrienes like LTC<sub>4</sub>, LTB<sub>4</sub> etc. Several cytokines also play essential roles in orchestrating the inflammatory process, especially interleukin-1 (IL-1) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). IL-1 and TNF are considered principal mediators of the biological responses to bacterial lipopolysaccharide (LPS, also called endotoxin). They are secreted by monocytes and macrophages, adipocytes, and other cells. Working in concert with each other and various cytokines and growth factors (including IL-8 and granulocyte-macrophage colony-stimulating they induce gene expression and protein synthesis in a variety of cells to mediate and promote inflammation. Prostaglandin (PGE<sub>2</sub>) or prostacyclin (PGI<sub>2</sub>) release increase blood flow as well as increase blood vessel permeability by assisting in release.

## MATERIALS AND METHODS

**Collection of samples and extraction:** The whole parts of *Acmella Oleracea* were collected from Herbal garden, Nehru Arts and science College, Coimbatore. The Leaves were washed under running tap water to eliminate dust and other foreign particles and to clean these thoroughly. The fresh Leaves, stem and flower were trodden into small pieces, powdered, and mixed in 1:10 ratio with distilled water and methanol separately. The extractions were obtained through continuous grind using mortar and pestle followed by filtration using Whatman No.1 filter paper. The residues were re-dissolved with the appropriate solvents for the further analysis.

**Phytochemical Components and Protein Determination:** Phytochemical analyses were carried out according to the methods described by Trease and Evans (1989). Protein content in the plant extracts were estimated by the method described by Lowry *et al.* (1951).

**Estimation of total Antioxidant** (Phosphomolybdenum method- Prieto *et al.*, 1999): Aliquot of 0.1 ml samples were obtained with 1 ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 mints. After the samples were cooled to room temperature, the absorbance of the aqueous solution of each tube was measured at 695 nm against blank, a typical blank solution contained 1ml of reagent solution and the appropriate volume of the same solvent used for the samples and it was incubated under same as rest of the sample. For sample of unknown composition, water soluble antioxidant capacity was expressed as equivalents of ascorbic acid (1 ml of extracts).

**Phenolic content as tannic acids** Jayaprakasha *et al.*, (2001): The extracts were dissolved in a mixture of methanol and water (6:4 v/v). sample (0.2ml) were mixed with 1 ml of tenfold diluted folin-ciocalteus reagents and 0.8 ml of 7.5% sodium carbonate solution, after standing for 30 mints at room temperature, the absorbance was measured at 765 nm. The estimation of phenolic compound in the fractions was carried out in triplicate and the results were averaged.

**Antibacterial activity:** *Klebsiella*, *Proteus*, *Shigella*, *Escherichia coli* and *Salmonella* were used for the study. Three or four isolated colonies were inoculated in the 2 ml nutrient broth and incubated till the growth in the broth was equivalent with Mac-Farland standard [0.5%] as recommended by WHO(2009). Antibacterial activity of the different extracts was determined by cup diffusion method on Muller Hinton agar medium by Anon (1996). Wells are made in Muller Hinton agar plate using cork borer [5 mm diameter] and inoculums containing bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. 20-80  $\mu$ l of the working suspension/solution of plant extracts and same volume of distilled water and methanol for

control were filled in the wells with the help of micropipette. Plates were kept for some time till the extract diffuse in the medium and incubated at 37°C for 24 h. After incubation, the plates were observed for the zone of inhibition [ZI], the diameter of the inhibition zone were measured and recorded.

#### Separation of Active Compound from Extracts from *Acmella Oleracea* Suspension by Thin Layer Chromatography (TLC)

**Preparation of chromaplate:** The glass slides were cleaned and dried in hot air oven. Slurry was prepared by mixing silica gel with double the volume of distilled water in a clean beaker. One drop of slurry was placed on the slide by using another slide edge, the drop of slurry was scattered all over to make thin film. The slides were kept as such for few minutes. Then the chromo plates were activated by heating in hot air oven at 120°C for 30 min. The slides were allowed to cool at room temperature and marked about 2 cm from the bottom as the origin. The working suspensions were loaded at the center of the each slide above from the edge.

**Development of chromatogram:** The development tank was saturated with suitable solvent according to Eskil Hultin (1966).

Alkaloids	: Benzene/ Methanol-80:20
Flavonoids	: Chloroform/Methanol-70:30
Lipid	: Chloroform/Methanol/water-10:10:3
Terpenoids	: Acetic acid/water-1:3

The slides were kept in the tank without touching baseline by solvent. The final solvent front was marked and the slides were dried.

**Spot visualization:** For visualization of Flavonoids 1% ethanolic solution of Aluminium chloride was used and viewed under 560nm UV light. Alkaloids and Terpenoids were visualized under UV light and they were visible as yellow and orange fluorescent spots. Few pieces of iodine crystals were kept in the tank and covered with glass plate to saturate the tank with iodine vapor for detecting lipids. The plate was then kept in iodine vapor saturated tank and left for few hours and brown colored spots were visualized.

**Retrieval of the active compound:** Spots on the preparative silica gel slides were scratched with the help of clean and dry spatula and collected in beaker containing appropriate solvents (Bishnu Joshi, 2011) and left overnight. The content in the beaker was stirred and filtrated through Whattsman no.1 filter paper. The filtrate was collected in clean and dry beaker. The filtrate containing active compound was used for the determination of antimicrobial effect against *Salmonella* and *Klebsiella* by cup diffusion method.

#### In vitro anti-inflammatory activity:

**Inhibition of albumin denaturation:** Methods of Sakat *et al.*, (2010) followed with minor modifications. The reaction mixture was consisting of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adjusted. The sample extracts were incubated at 37°C for 20 min and then heated to 51°C for 20 min. after cooling the samples the turbidity was measured by spectrophotometrically at 660nm. The experiment was performed in triplicate. Percent inhibition of protein denaturation was calculated as follows,

$$\%inhibition = \frac{Abs\ control - Abs\ sample}{Abs\ control} \times 100$$

Where Abs control is the absorbance without sample, Abs sample is the absorbance of sample extract/standard.

#### Membrane stabilization test:

**Preparation of red blood cells (RBCs) suspension:** Fresh whole human blood (10ml) was collected 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 (Sadique, 1989; Saket, 2010)

**Heat induced haemolytic:** The reaction mixture (2 ml) consisted of 1 ml of test samples solution and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Ibuprofen was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56°C for 30min. At the end of the incubation the tubes 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 experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formula mentioned above (Shinde *et al.*, 1999; Saket *et al.*, 2010)

**Protein inhibitory action:** The test was performed according to the modified method of Oyedepo *et al.*, (1995) and Sakat *et al.*, (2010). The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1ml of 20mM Tris HCl buffer (pH7.4) and 1ml test sample of different concentrations of different solvents. The reaction mixture was incubated at 37°C for 5min and then 1ml of 0.8% (W/V) casein was added. The mixture was inhibited for an additional 20 min, 2ml of 70% perchloric acid was added to terminate the reaction. Cloudy suspension was centrifuged and the absorbance of the supernatant was read at 210nm against buffer as blank. The experiment was performed in triplicate. The percentage of inhibition of proteinase inhibitory activity was calculated.

## RESULTS

**Phytochemical analysis and protein content:** Qualitative phytochemical analyses were performed according to the methods described by Trease and Evans (1989) for the detection of alkaloids, saponin, steroids, flavonoids, cardiac glycosides, cynogenic glycosides and phlobatannins. The observed results were tabulated (Table 1). Protein content in the plant extracts were estimated by the method described by Lowry *et al.* (1951). 0.02 ml and 0.04 ml of aqueous and methanol extracts were used to determine the protein content. Standard graph was plotted for bovine serum albumin using spectrophotometric reading at 660 nm and it contained 2.6 mg/g, 1.6mg/g and 2.8mg/g of protein in aqueous extract of the leaves, stem and flower. The result was tabulated in table 2.

**Total anti-oxidant and Phenolic content:** The absorbance of the aqueous and methanol solution of each tube was measured for total anti-oxidant content at 695 nm against blank it was observed that leaves contain 14.21 mg/g and 11.22 mg/g of anti-oxidant in methanol and aqueous extract. Stem contained 5.68 mg/g and 5.04 mg/g of anti-oxidant content in both methanol and aqueous extract. Flower contained 5.62 mg/g and 3.51 mg/g of anti-oxidant content in both methanol and aqueous extract. The absorbance was measured at 765 nm for Phenolic content. The estimation of phenolic compound in the fractions was carried out in triplicate and the results were averaged. Leaves contain 11.72 mg/g and 12.32 mg/g of phenolic content in methanol and aqueous extract.

Stem contained 6.37 mg/g and 5.18 mg/g of phenolic content in both methanol and aqueous extract. Flower contained 5.67 mg/g and 4.58 mg/g of phenolic content in both methanol and aqueous extract. The results were tabulated table 3.

**Antibacterial Property:** Minimal inhibitory concentration assay was carried out for methanolic extraction *Acmella Oleracea* *Shigella*, *Proteus*, *Klebsiella*, *Escherichia coli* and *Salmonella*. Antibacterial properties of the *Acmella Oleracea* extracts were carried out by well diffusion method as described by Anon (1996). The methanol extract of *Acmella Oleracea* leaves showed 2 cm the maximum zone of inhibition in *Proteus* followed by *Salmonella typhi* and minimum zone of inhibition found in methanol extract in *Escherichia coli* and methanol extracts of stem showed maximum zone of inhibition found in *Escherichia coli* followed by *Proteus* which is tabulated in table 5.

**Separation of Active Compound from Thin Layer Chromatography (TLC):** Thin layer chromatography techniques were carried out using respective solvents as mentioned in the materials and methods in order to detect the presence of alkaloid, flavonoids, lipids and terpenoids. Table 6 represented the methanol extract of leaf and stem contain alkaloids, flavonoids, lipids and terpenoids in the Rf value of 0.45, 0.5, 1.2 and 0.66. The flower contained the Rf value of 0.5, 0.46, 1.5, 0.72. The separated active compounds alkaloid, flavonoids, lipids, and terpenoids were found that effective against *Salmonella* and *Klebsiella* the zone of inhibition tabulated in table 7 which represented the maximum zone of inhibition of leaves extract found in *Klebsiella* in the active compound of flavanoids and minimum zone of inhibition found in active compound of lipid (Fig 1).

#### **In-vitro anti-inflammatory activity:**

**Inhibition of albumin denaturation:** Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation (Table 8). Maximum inhibition was observed from leaf extract followed by stem and flower. Ibuprofen, a standard anti-inflammation drug showed the maximum inhibition 56.4% at the concentration of 200 µg/ml.

**Membrane stabilization test:** Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of aqueous extract of *Acmella Oleracea*. The extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree (Table 8). The Ibuprofen standard drug standard drug showed the maximum inhibition 57.6%.

**Proteinase inhibitory activity:** The *Acmella Oleracea* aqueous extract exhibited significant anti proteinase activity from different parts. The maximum inhibition was observed from flower ethanolic extract, in decreasing order was stem and flower aqueous extract. The standard Ibuprofen drug showed the maximum proteinase inhibitory action (Table 8).

#### **DISCUSSION**

The presence of alkaloids, saponin, steroids, flavonoids, cardiac glycosides and cynogenic glycosides. The aqueous and methanolic extract of both leaves, stem and flower of *Acmella Oleracea* contain alkaloids, saponin, steroids, flavonoids, cardiac glycosides and cynogenic glycoside. A qualitative phytochemical analysis was performed for the detection of secondary plant metabolites [viz., alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins] and reducing sugars (Susmitha, 2013). The methanol extract of *Acmella Oleracea* leaves showed 2 cm the maximum zone of inhibition in *Proteus* followed by *Salmonella typhi* and minimum zone of inhibition found in methanol extract in *Escherichia coli* and methanol extracts of stem showed maximum zone of inhibition found in *Escherichia coli* followed by *Proteus*.

The antibacterial activity of aqueous, ethanol and acetone extracts of *Corriander sativum*, *Abutilon indicum*, *Boerhavia diffusa* and *Rograpis paniculata*, *Plantagoovata*, *Euphorbia ligularia*, *Zinziberofficinale*, *Terminalia chebula*, *Azadirachta indica*, *Ocimum sanctum* and *Cinnamomum cassia* was determined against 33 UTI isolates i.e. *Proteus mirabilis*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Providencia pseudomallei*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca* by disc diffusion method. The studies concluded that crude extracts of the selected plants especially the acetone and ethanol extracts exhibited significant activity against UTI pathogens (Anjana Sharma, 2011).

Alcoholic extracts of stem of an endangered medicinal plant *Spilanthes acmella* and its *in vitro* raised callus were evaluated for antibacterial potential against various gram positive and gram negative bacteria including resistant isolates harbouring *bla* genes by agar well diffusion method. The alcoholic extract of parent plant as well as its callus showed good antibacterial activity against gram positive and gram negative bacteria and also efficiently controlled the growth of most of the resistant bacteria harbouring *bla* genes. Minimum inhibitory concentrations (MIC) of the extracts was determined by broth microdilution method. MIC against gram positive bacteria ranged from 12.0 to 49.0 µg/ml, while MIC against gram negative bacteria ranged from 1.53 to 12.0 µg/ml and MIC against resistant bacteria harbouring *bla* genes ranged from 6.1 to 98.0 µg/ml (Noor Jahan, 2013).

The methanol extract of leaf and stem contain alkaloids, flavonoids, lipids and terpenoids in the Rf value of 0.45, 0.5, 1.2 and 0.66. The separated active compounds alkaloid, flavonoids, lipids, and terpenoids were found that effective against *Salmonella* and *Klebsiella* the zone of inhibition. Chromatographical analysis of *Acmella Oleracea* revealed that the presence of terpenoids, lipids, alkaloids and flavanoids. Chromatographic profiles of crude extracts obtained through different solvents were similar. The visualization of chromatographic profiles for each extraction technique and solvent used permit to evaluate the qualitative and quantitative variations in secondary metabolites content by Cristiane 2009. In addition, these data present compound profiles related to the biological effects and medicinal use.

An attempt was made to review the basic principles and the importance of Thin Layer Chromatography (TLC) in research in general and in phytochemistry in particular. Thin layer chromatography is a simple, cost-effective, and easy-to-operate planar chromatographic technique which has been used in general chemistry laboratories for several decades to routinely separate chemical and biochemical compounds. Traditionally, chemical and optical methods are employed to visualize the analyte spots on the TLC plate. Also it has a wide application in identifying impurities in a compound. Study highlights the review on TLC and its

application of qualitative and quantitative estimation of bio-active compounds from medicinal plants (Sanjeet Kumar, 2013).

Denaturation of proteins is a well-documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of extract protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition was observed from leaf extract followed by stem and flower. The active compounds obtained from the plant part also had more or less anti-inflammatory activity. Ibuprofen, a standard anti-inflammation drug showed the maximum inhibition 0.564% at the concentration of 200 µg/ml.

Stabilization of RBCs membrane was studied for further establishes the mechanism of anti-inflammatory action of aqueous extract of *Acmella Oleracea*. The extracts were effectively inhibiting the heat induced hemolysis. These results provide evidence for membrane stabilization as an additional mechanism of their anti-inflammatory effect. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. The extracts inhibited the heat induced hemolysis of RBCs to varying degree. Eugenol (1-hydroxy-2-methoxy-4- allylbenzene) a naturally occurring phenolic compound is a major component of basil oil and exists to a lesser extent in oil of several other plants (Nagababu *et al.*, 1995). It possesses anti-ulcer, anti-septic, analgesic, anti-bacterial, anti-inflammatory and anti-anaphylactic properties (Nagababu, 1995; Prakash and Gupta, 2005). The therapeutic potential effect of the fresh leaves of *Ocimum sanctum L.* has been found to be largely due to eugenol. Nitin gupta, *et al.*, 2012 reported that the anti-inflammatory activity of spilanthos, For centuries, *Spilanthos acmella (L.)* Murr. (Fam. Compositae) has been recommended in traditional medicine for treatment of toothache, rheumatism and fever.

**Table.1.Phytochemical analysis of *Acmella oleracea***

Phytochemical constituents	Aqueous extract			Methanol extract		
	leaves	stem	flower	leaves	stem	flower
Alkaloids	+	+	+	+	+	+
Steroids	+	+	-	+	+	-
Saponin	+	+	+	+	+	+
Cynogenic glycosides	+	+	-	+	+	+
Cardiac glycosides	-	-	-	+	+	+
Flavonoids	+	+	+	+	+	+
Phlobatannins	-	-	+	-	-	+
+Phenols	+	+	+	+	+	+

‘+’ – Present

‘-’ – Absent

**Table.2.Total protein content of *Acmella oleracea***

Samples	Concentration of proteins (mg/g)		
	Leaf	Stem	flower
Aqueous extract	2.6	1.6	2.8
Methanol extract	8.2	3.2	5.6

**Table.3.Total anti-oxidant and phenol content of *Acmella oleracea***

Sample	Anti oxidant content OD at 695 nm (mg/g)			Phenolic content OD at 765 nm(mg/g)		
	Leaf	stem	flower	Leaf	stem	flower
Methanol extract	14.21	5.68	5.62	11.72	6.37	5.67
Aqueous extract	1.122	0.504	3.54	12.32	5.18	4.58

**Table 4. MIC assay (methanol extraction) of *Acmella oleracea***

Test organisms	Leaf (OD at 540 nm)			Stem (OD at 540 nm)			Flower (OD at 540 nm)		
	60µl	80µl	100µl	60µl	80µl	100µl	60 µl	80 µl	100 µl
<i>Salmonella typhi</i>	0.48	0.32	0.15	0.29	0.19	0.27	0.22	0.18	0.8
<i>pseudomonassp.</i>	0.40	0.34	0.12	0.42	0.14	0.09	0.31	0.22	0.10
<i>Escherichia coli</i>	0.58	0.52	0.44	0.16	0.10	0.06	0.24	0.18	0.11
<i>Klebsiellasp.</i>	0.77	0.22	0.17	0.31	0.18	0.08	0.32	0.24	-
<i>Shigella sp.</i>	0.53	0.21	0.09	0.28	0.11	0.05	0.36	0.28	0.16

**Table.5.Antimicrobial activity of methanolic extract of *Acmella oleracea***

Test organisms	Zone of inhibition(in diameter in cm)												
	Leaf				Stem				Flower				Standard drug (amp)
	20µl	40µl	60µl	80µl	20µl	40µl	60µl	80µl	20 µl	40 µl	60 µl	80 µl	
<i>Salmonella typhi</i>	0.8	1.4	1.3	1.7	-	-	-	-	-	-	-	-	1.2
<i>Shigella sp.</i>	-	1.1	1.2	1.5	-	-	-	-	-	1	1.2	2	2
<i>Escherichia coli</i>	-	1.2	1.4	1.6	-	-	-	1.2	-	1.2	1	2.1	2
<i>Proteus sp.</i>	-	1.2	1.6	2.0	-	-	-	1	0.6	1	1.3	2.2	1
<i>Klebsiella sp.</i>	-	0.7	1	1.5	-	-	-	-	-	-	-	-	-

**Table.6.Rf values of phytochemicals of aqueous extract *Acmella oleracea***

Sample	Flavonoids	Alkaloids	Lipids	Terpenoids
Leaf	0.4	0.41	1.3	0.68
Stem	0.4	0.41	1.3	0.68
Flower	0.5	0.46	1.5	0.72

**Table.7. Antimicrobial activity of active compounds of *Acmella oleracea* (methanolic extract)**

Active compounds	Plant part	Zone of inhibition (in diameter mm)	
		Test organism	
		<i>Salmonella sp.</i>	<i>Klebsiella sp.</i>
Lipid	Leaf	1.7	2.1
	Stem	1.8	1.9
	Flower	1.6	2
Flavonoids	Leaf	2.1	2.5
	Stem	2.2	2.1
	Flower	1	1.5
Alkaloids	Leaf	2.4	2.1
	Stem	1.8	1.9
	Flower	1	1.4
Terpenoids	Leaf	2.2	2.1
	Stem	2.2	2
	Flower	-	-

**Table.8. Effect of water extracts of *Acmella oleracea* on albumin denaturation, membrane stabilization and proteinase inhibitory activity percentage inhibition**

Active compounds	Plant part	Albumin denaturation	Membrane stabilization	Proteinase inhibition
Lipid	Leaf	10%	10.1%	10%
	Stem	8%	9%	9.1%
	Flower	7%	7.4%	7.4%
Flavonoids	Leaf	8.2%	9.1%	9%
	Stem	7.1%	7.3%	7%
	Flower	6%	5.8%	5.2%
Alkaloids	Leaf	8.7%	9.1%	9%
	Stem	6.1%	6.3%	7%
	Flower	6.9%	6.8%	6.2%
Terpenoids	Leaf	7.8%	7.1%	7.2%
	Stem	6.5%	6.3%	6.8%
	Flower	5.9%	5.4%	5.1%
Aqueous extract	Leaf	38%	38.2%	38.1%
	Stem	36.5%	36.5%	36.3%
	Flower	32.2%	33%	32.1%
<b>Ibuprofen (200 µg/ml)</b>		54.6%	57.6%	58.2%

*Proteus**Salmonella***Figure.1. Antimicrobial activity of active compounds**

## CONCLUSION

Plant essential extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine, and natural therapies. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In-vitro* studies in this work showed that the *Acmella Oleracea* plant extracts inhibited bacterial growth and have anti-inflammatory activity, but their effectiveness varied depend upon the metabolic compounds present in the plant.

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## REFERENCES

- Anjanasharma, S., Chandraker, V.K., Patel, and Padmini Ramteke, Antibacterial activity of medicinal plants against pathogens causing complicated tract infections, *Indian journal of pharmaceutical Sciences*, 71, 2011, 136-139.
- Bishnu Joshi, Phytochemical extraction and antimicrobial properties of different medicinal plants. *Journal of Microbiology and Antimicrobials*. 3(1), 2011, 1-7.
- Eskil Hultin, Thin layer chromatography of plant extracts. *Acta chemica scandinavica*, 20, 1996, 1588-1592.
- Kumar S, Bajwa BS, and Singh Kuldeep, Anti-Inflammatory Activity of Herbal Plants. *International journal of advances in Pharmacy*. 2(2), 2013, 2277 – 4688.
- Nitin gupta, Ankit, R., Patel and Ravindra, R. P, Design of akkalkara (*Spilanthes acmella*) formulations for antimicrobial and topical anti-inflammatory activities. *International Journal of Pharma and Bio Sciences*. 2012, 161–170.
- Noor Jahan, Razia Khatoon, Siraj Ahmad, and Anwar Shahzad, Evaluation of antibacterial potential of medicinal Plant *Spilanthes acmella* Murr. and its in vitro raised callus against resistant organisms. *Journal of Applied Pharmaceutical Science*. 3(10), 2013, 119-124.
- Oyedapo, O.O., Famurewa, A.J, Antiprotease and membrane stabilizing activities of extracts of *Fagara Zanthoxyloides*, *Olox subscorpioides* and *Tetrapleura tetraptera*. *Int. J. Pharmacogn*. 33, 1995, 65– 69.
- Prakash, P., and Gupta, N, Therapeutic uses of *Ocimum sanctum* Linn (tulsi) with a note oneugenol and its pharmacological actions: a short review, *Indian J. Physiol. Pharmacol*, 49 (2), 2005, 125 131.
- Prieto, P., Prenda, M., and Aguilar, M, Spectrophotometric quantification of antioxidant capacity though the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem*. 269, 1999, 337-341.
- Sadique J, Al-Rqobahs WA, Bughaith El-Gindi Ar, The bioactivity of certain medicinal plants on the stabilization of RBS membrane system. *Fitoterapia*, 60, 1998, 525-532.
- Sakat S, Juvekar AR, Gambhire MN, In vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *I. J. Pharm. Pharm. Sci.*, 2(1), 2010, 146-155.
- Sanjeet Kumar K, Jyotirmayee, Monalisa Sarangi, Thin layer chromatography. *International Journal of Pharmaceutical Sciences Review and Research*, 2013, 34-45
- Shashank Matthew, Ajay Kumar Jain, Manoj James, Cathrin Matthew and Debjit Bhowmik. 2013. Analgesic And Anti-Inflammatory Activity of *Kalanchoe Pinnata* (Lam.) Pers. *Journal of medicine plant studies*. 24- 28.
- Shinde UA, Phadke AS, Nari AM, Mungantiwar AA, Dikshit VJ, Saraf MN, Membrane stabilization activity- a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*, 70, 1990, 251-257.
- Susmitha, S., Vidyamol, K.K., Ranganayaki, P., and Vijayaragavan, R, Phytochemical Extraction and Antimicrobial Properties of *Azadirachta indica* (Neem). *Global Journal of Pharmacology*, 2013, 316-320.