

## Research Article

# Investigation of the Anti-Microbial and Anti-Inflammatory Effect of *Cyperus Rotundus* on Tonsillitis

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## Abstract

Since times immemorial people have been using herbs or herbal extracts in the form of medicine. One such medicinal plant being, *Cyperus rotundus* is a waste weed belonging to the family Cyperaceae, which is a native of Africa, Southern and Central Europe and Southern Asia. The plant has now been identified as possessing several pharmacologically active substances such as  $\alpha$ -cyperone,  $\beta$ -selinene, cyperene, cyperotundone, patchoulone, sugeonol, kobusone, and isokobusone. A thorough scientific study should explain the folk- and alternative medicinal uses and the present study is therefore aimed at the evaluation of antimicrobial & anti-inflammatory activity of *Cyperus rotundus* for tonsillitis. The ethyl alcohol extract of *Cyperus rotundus* was investigated for antibacterial activity against *Streptococcus pyogenes* responsible for throat infection. After an incubation of 48 hours, a prominent clear inhibition zone was observed in the plate containing *Cyperus rotundus* extract. The inhibition zone test was followed by biopsy studies in which 16 tonsillectomy samples were collected in order to study the different organisms responsible for tonsillitis. Zymography was used to check the levels of reduction in inflammation by analyzing the activity of MMP (Matrix Metalloproteinase). The 16 samples subjected to this test revealed a difference in the levels of MMP-2 and MMP-9 expression. After treatment the reduction in the levels of MMP in all samples ranged from 20% to 90%. Therefore, from the tests it was clear that the extract used for tonsillitis has a broad range of antimicrobial action and that it is an effective drug for treating tonsillitis.

**Keywords:** Antimicrobial, Anti-inflammatory, *Cyperus rotundus*, Tonsillitis, Matrix metalloproteinase (MMP).

## 1. Introduction

Tonsillitis is the inflammation of the tonsils. The complications of acute streptococcal tonsillitis are proportional to the severity of the infection. The infection may extend upward into the nose, sinuses, and ears or downward into the larynx, trachea, and bronchi. Characteristic symptoms include pharyngeal pain and dysphagia, sometimes accompanied by fever. Although the majority of cases of tonsillitis are viral in origin, Bacterial infection is the second main cause of tonsillitis. The most common bacterial cause is Group A  $\beta$ -hemolytic streptococcus (GABHS), which causes strep throat. Less common bacterial causes include: *Staphylococcus aureus* (including methicillin resistant *Staphylococcus aureus* or MRSA), *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, *pertussis*, *Fusobacterium*, *diphtheria*, *sypilis* and *gonorrhoea*.. Currently used options for treatment of GABHS tonsillitis are far from optimal, thus creating the need for new agents which are antimicrobial that combine short course therapy for patient convenience with activity against increasingly

prevalent macrolide-resistant strains of *S. pyogenes* (James Quinn et al, 2003)) since *Streptococcus pyogenes* (group A streptococcus, GAS) is an important Gram-positive human pathogen, which causes mild to severe diseases, including tonsillitis, pharyngitis, cellulitis, scarlet fever, rheumatic fever, necrotizing fasciitis, and streptococcal toxic shock syndrome (STSS)(Chunan Chiang et al, 2008). The search for safe and effective natural antibacterial is now focused on plants. *Cyperus rotundus* is one such plant and the most invasive weeds known, having spread out to a worldwide distribution in tropical and temperate regions possessing anti-bacterial property for a wide spectrum of micro-organisms. Food related bacteria like *Staphylococcus aureus*, *enterococcus faecalis*, *escherichia coli* and *salmonella typhimurium* were found to be affected by the antibacterial activity of *Cyperus rotundus* extracts (Soumaya kilania et al, 2008).

Matrix metalloproteinases (MMPs) are associated with various physiological processes such as morphogenesis, angiogenesis, and tissue repair. MMPs are a family of calcium-dependent, zinc-containing endopeptidases that are structurally and functionally related. tightly regulated by Tissue inhibitors of metalloproteinases (TIMPs). MMPs proteolytically activate or degrade a variety of nonmatrix

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substrates including chemokines, cytokines, growth factors, and junctional proteins. Thus, they are increasingly recognized as critical players in inflammatory response. MMP-2, MMP-7, MMP-9 and tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) due to dysregulated turnover of connective tissue matrices in children causes recurrent tonsillitis (Engin Acioglu et al, 2010).

MMPs are considered as viable drug targets in the therapy of the inflammatory diseases. MMP's are substrate specific and with gelatin as substrate two MMP's are grouped as gelatinases i.e gelatinase A (MMP-2) and gelatinase B (MMP-9) (Emma Pirila et al, 2001). The balance between MMPs and their inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), is largely responsible for the remodeling of tissues. Zymographical techniques are used for the analysis of MMPs and TIMPs. MMPs can be analyzed with several zymographical techniques, but substrate zymography is the most commonly used (K. Kupai et al., 2010).

## 2. Material & Methods

### 2.1 Collection of plant material:

The *Cyperus rotundus* rhizomes samples were collected from the fields of Vadgaon, Belgaum.

### 2.2 Preparation of plant powder

The rhizome sample was dried using hot air oven at a temperature of 52 ° C and was finely grinded to powdered form using mortar and pestle.

### 2.3 Alcoholic extraction of phytochemicals from *Cyperus rotundus*

27gms dried fine sample was subjected to soxhalation in ethanol solvent for 28hours. The temperature was maintained at 72 ° C. The solvent was removed by distillation under reduced pressure and the resulting semi-solid mass was dried at room temperature to obtain the extract.

### 2.4 Antibacterial activity

*Streptococcus pyogens* were inoculated in plates containing MRS agar media & incubated at 37 ° C for 48 hrs. The disc was dipped in extract of *Cyperus rotundus* & Similarly another disc was dipped in alcohol (Control) . To check the antimicrobial activity the disc was kept onto inoculated plates and was incubated for 48 hours. After the incubation period each plate was observed for zone of inhibition.

### 2.5 Collection of tonsillitis sample

The tonsil sample for biopsy study and the microbial plating was collected from the children of age 8-15years from Civil Hospital, Belgaum.

### 2.6 Detection of MMP-2 and MMP-9 levels on tonsillitis samples using gelatin zymography.

The detection of MMPs activity is done by the substrate zymography (K. Kupai et al, 2010). This is an electrophoresis technique, based on SDS-PAGE that includes a substrate copolymerized with the polyacrylamide gel, for the detection of enzyme activity based on molecular weight separation. The active and the latent forms of the MMPs can be visualized because they have a different molecular weight. The most commonly used substrate is gelatin for MMP-2 and MMP-9.

## 3. Result and discussion

### 3.1 Antibacterial activity

The results of the above performed procedures were in favor of *Cyperus rotundus* as a drug for treatment of tonsillitis. Extraction done using the conventional mortar-pestle was used to check the inhibition zone. Since *Streptococcus pyogens* are identified to be one of the species causing infection in throat leading to tonsillitis, the growth inhibition of this species with the rhizome extract was checked. After 48 hours, a prominent clear inhibition zone was observed in the plate containing *Cyperus rotundus* extract. As control, *S. pyogens* was cultured with ethanol disc. Following figure shows the inhibition zones after incubation for 48 hours:



**Figure 1:** Inhibition zone of extract of *Cyperus rotundus* Diameter of inhibition zone= 2 cm.



**Figure 2:** Inhibition zone of alcohol Diameter of inhibition zone=1.2cm



**Figure 3:** Inhibition zone of extract of *Cyperus rotundus* after 72 hours. Diameter of inhibition zone=0.7cm

It is clearly seen that there is a clear inhibition zone in case of plate with *Cyperus rotundus* extract. The diameter of inhibition zone of *Cyperus rotundus* extract is more than the inhibition zone of control. Thus, it is understood that effect of ethanol accompanied by presence of phytochemicals of *Cyperus rotundus* gives a bigger and

**Table1:** Levels of MMP-2 and MMP-9 before and after treatment of specimen with extracts of *Cyperous rotundus*.

S. No.	Sample name	Age	Bands		% of reduction*	
			MMP-9	MMP-2	MMP-9	MMP-2
1.	Patient1	9	Yes	Yes	50%	70%
2.	Patient2	11	Yes	Yes	same	same
3.	Patient3	13	Yes	Yes	70%	90%
4.	Patient4	10	Yes	No	same	no
5.	Patient5	10	Yes	Yes	40%	40%
6.	Patient6	12	Yes	Yes	60%	80%
7.	Patient7	9	Yes	Yes	50%	60%
8.	Patient8	14	Yes	No	50%	No
9.	Patient9	12	Yes	Yes	Same	Same
10.	Patient10	13	Yes	Yes	20%	20%
11.	Patient11	10	Yes	Yes	30%	Same
12.	Patient12	11	Yes	Yes	50%	80%
13.	Patient13	15	Yes	Yes	50%	70%
14.	Patient14	13	No	Yes	no	90%
15.	Patient15	12	No	No	no	No
16.	Patient16	10	No	Yes	no	90%

\*: % of reduction- is the reduction in the size and intensity of the band after the treatment of the sample with the given compound.

prominent inhibition zone and hence the growth of microbe (*S. Pyogens*) is inhibited. The results not only showed a clear zone but also sustained the zone for a longer time. When the plates were further incubated for another 24 hours, the inhibition zone in case of *Cyperous rotundus* was still seen. Although the diameter decreased, the clarity of zone was the same. On the other hand, the plate which served as control could not sustain the inhibition zone. This implies that *Cyperous rotundus* had a longer inhibition effect on *Streptococcus pyogens*.

### 3.2 Anti inflammatory activity

To check the actual levels of reduction in inflammation, the levels of Matrix metalloproteinase (MMP) were checked by gelatin Zymography. The 16 samples subjected to this study showed different levels of MMP-2 and MMP-9 expression on them. Table1 shows the levels of MMP-2 and MMP-9 before and after treatment of specimen with extracts of *Cyperous rotundus*. MMP-2 and MMP-9 are essential for the degradation of the collagen IV, which are the most important component of the Extra Cellular Matrix (ECM) degradation during tissue hypertrophy and remodeling in the superficial part and core regions of the tonsillar tissue. (Engin Acioglu et al, 2010). Control was considered so as to check for the effect of ethanol onto the specimen. Sample number 2 and 8 (table 1) represent control samples. Bands obtained before the treatment of ethanol were similar to the one's after treatment. The levels of MMP have gone down from 20% to 90% in some cases. This clearly showed that ethanol had no effect on MMP levels. This concludes that *Cyperous rotundus* has proved to be a successful in reduction of MMP-2 and MMP-9 levels thus indirectly affecting the inflammation level of tonsillitis.

### Conclusion

The extract of *Cyperus Rotundus* can be a potential treatment for tonsillitis. There was a clear zone of inhibition observed which suggests the anti bacterial activity of the *Cyperus rotundus*. Further, the reduction in level of MMP2 & MMP 9 confirms the anti-inflammatory activity of *Cyperus rotundus*.

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