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Evaluation of Anti-Septic and Anti-Inflammatory Activity of *Elytraria acualis.*

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Abstract: Inflammation is a body defense reaction to prevent the spread of injurious agent and to remove them across the cells and tissues. Inflammatory abnormalities are a large group of disorders which underlie a vast variety of human diseases. During treatment of inflammatory diseases, many conventional therapies (non-steroidal anti-inflammatory drugs) are used to relief pain and inflammation. If any foreign particle enters through the wound, cut or burns it causes inflammation and later it causes septicaemia. Continuous use of the intended drugs is frequently associated with serious side effects, whereas plants still hold their own unique place, by the way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation so as to exploit them as herbal anti-inflammatory and anti-septic agents with a better safety profile.*Elytraria acualis* has many therapeutic uses mentioned in Ayurveda and therefore we aimed to study its anti-inflammatory and anti-septic activities of *Elytraria acualis.*
Key words: Anti-inflammatory, Anti-septic activity, *Elytraria acualis,* ethanolic extract.

1. Introduction:

Inflammation is defined as the local response of living mammalian tissues to injury due to any agent. It is a body defence reaction to prevent the spread of injurious agent and to remove them across the cells and tissues. The development of non-steroids in overcoming human sufferings such as Rheumatoid arthritis has evoked much interest in the extensive search for new drugs with this property. 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. 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. Inflammation causes severe disorders such as Acne vulgaris, asthma ,Autoimmune diseases, Hypersensitivities, Transplant rejection, Rheumatoid arthritis. In this research we are going to evaluate the anti-septic and anti-inflammatory activities of *Elytraria acaulis.*

Elytraria acualis[1-10]*:*

This belongs to family Acanthaceae, which is a small shrub, which grows in shady dry places. In Tamil it is called as Nilakadambu. This plant is traditionally used for healing of wounds.The phytochemical screening of ethanol extract shows the presence of chemical compound like Alkaloids, Flavonoids, Protein, Amino Acid, Glycosides, Carbohydrates, Phenol, Steroids, Saponins and Tannins. The chromatographic studies give various spots (HPTLC) with methanol extract may confirm the presence of alkaloid contents in the plant. *Elytraria acaulis*is widely distributed in South Africa and India. *Elytraria acaulis* is traditionally used in the treatment of asthma, migraine, leucorrhoea, snake bite. The *Elytraria acaulis* extracts were effective in decreasing blood glucose level, increases oral glucose tolerance test, moderately alteration in body weight and there was a marked reduction in the liver glycogen levels and reduction in glycated hemoglobin levels. Histopathological study, showing improvement with nearly normal islets of langerhans, showing marked improvement with normal architecture with mild hepatocytes degeneration and showing acid significantly inhibited glomerular hypertrophy, glomerulosclerosis.

2. Materials and Methods[5-23]:

Qualitative and Quantitative analysis were done and the ethanolic extracts of the two plant species was used for GC-MS studies. The details of the material used and methods followed are described below.

2.1 Collection of plant materials and extraction

Fresh plant sample of *Elytrariaacualis* (except root whole plant is used) was collected from the villupuram district of drought area (this plant found all over the South Asia). The sample was collected and dried under shady region and it is grinded mechanically. The plant materials were powdered and 20gm of powder sample was extracted with 200ml of ethanol (1:10) by using soxhlet apparatus. The extract was concentrated to dryness and the residues were transferred to a preweighed sample bottle and were stored in a desiccator for further studies.

2.2.Qualitative analysis

The presence of reducing sugars, flavonoids, terpenoids, steroids, tannins, saponin, glycosides, alkaloides were estimated using the standard analytical methods such as Fehling's test, Shinoda test ,Libermann-Burchard test, Ferric chloride test, Foam test, Killer-Killiani test, Mayer's test.

2.2.1. Test for Anthraquinones

To the powdered material 10 ml of 1% HCl was added and this mixture is boiled for 5 minutes in a boiling water bath. Filter the sample and allowed to cool. Partition the cool filtrate against equal volume of chloroform. Carefully transfer the chloroform layer into clean test tubes. Shake with equal volume of 10% ammonia solution and allow the layer to separate. Presence of delicate rose pink colour indicates the presence of combined anthraquinones.

2.3. Quantitative test for *Elytrariaacualis*

2.3.1. Determination of moisture

5 gm of material was taken in a pre-weighed petridish. The petridish was placed without lid into an oven at 110^0C for three hours. The petridish was taken out and closed immediately with a lid. The dish was cooled in a desiccator and weighed. The amount of moisture of the material was calculated from the difference in weight.

2.3.2. Total carbohydrate

Weighed amount of fresh tissue was homogenized with distilled water. The homogenate was filtered using a two layered cheese cloth. The filtrate was then centrifuged at 10,000rpm for 15min. The supernatant was collected and the volume was made up to 25ml using distilled water. An aliquot of sample was pipetted out and 4ml Anthrone reagent added. It was then kept in a boiling water bath for 10 min. The tubes were cooled and the absorbance was measured at 530nm. The amount of total carbohydrate present was determined using the standard graph of glucose.

2.3.3. Estimation of protein

Total protein present in the plant was estimated by Lowry's method. 1gm powdered plant material was homogenized in 5ml of 0.1 M PO_4 buffer. The homogenate was filtered through double layered cheese cloth and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and the volume was made up to 1.5ml by PO_4 buffer. After that 1.5ml of Bradford reagent was added and kept it for 5 minutes. The absorbance was recorded spectrophotometrically by using appropriate blank at 595nm.The protein content was calculated from the standard graph of BSA or Bovine Serum Albumin.

3. Analysis of the Sample:

The sample was analysed using GC-MS (for identification of compounds), Anti-bacterial testing for this Agar well diffusion and disk diffusion methods were employed. This is to study the zone of inhibition which is used to study the anti-septic property of the extract.

4. Result

The qualitative phytochemical investigations of *Elytrariaacualis* Linn extract showed the presence of steroids, Flavonoids, Saponins, alkaloids and tannin in the ethanol extracts. Results showed the moisture content of plant was found to be 90%, while the least content was found to be phenol which was only about 0.002mg/g fresh tissue.

4.1. Quantitative analysis of *Elytrariaacualis*

Phtochemical analysis of *Elytrariaacualis* is given in table 1.

Table 1: Phytochemical of *Elytrariaacualis*

Test	Test method	Test result
Flavanoid	Shinoda test	+
Glycoside	Killer-killiani test	+
Alkaloid	Mayers test	+
Tannin	Ferric chloride	+
Saponin	Foam test	+
Anthroquinone		+
Steroids	Libermann.buurchard test	+
Terpenoids	Libermann.buurchard test	+
Reducing sugar	Fehlings test	+

4.2. GC-MS result:

GC-MS chromatogram analysis of the ethanolic extract of *Elytrariaacualis* shows the peak value about 15.82 (Fig. 1). There are 14 components out of 19 shows the anti septic and anti inflammatory activity in the table 2.

Fig.1. GC-MS Result

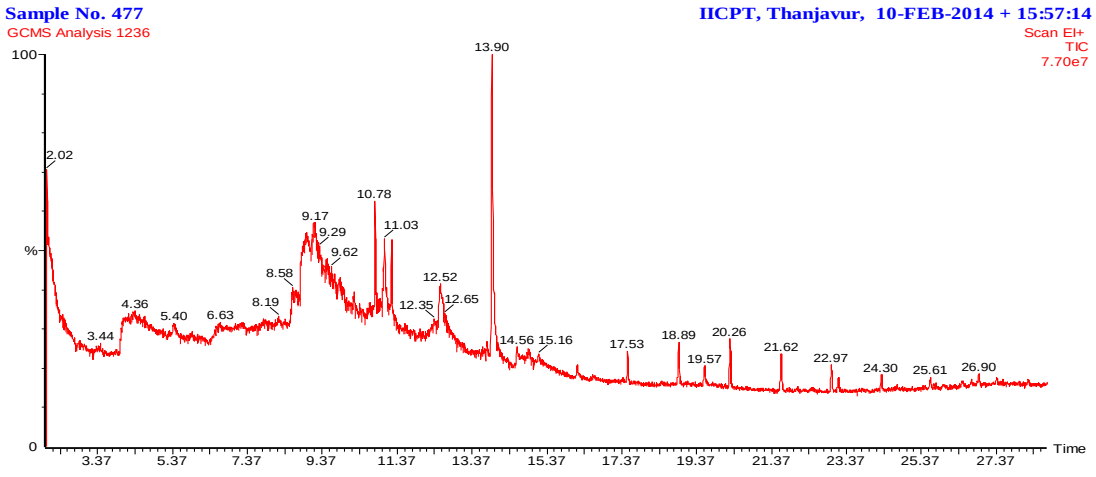


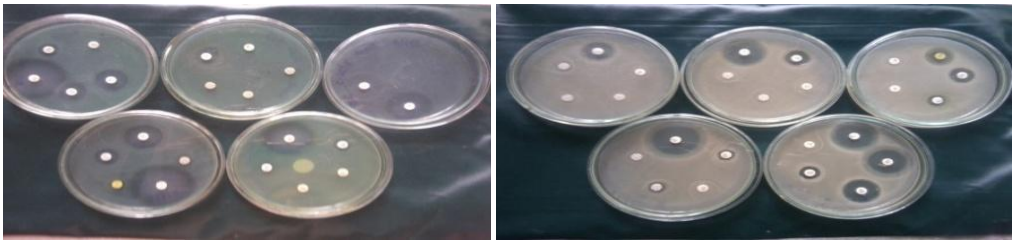
Table.2.Compounds identified in *Elytrariaacualis*

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	4.36	2(3H)-Furanone, dihydro-4-hydroxy-	$C_4H_6O_3$	102	7.90
2.	5.40	Butanal, 3-hydroxy-	$C_4H_8O_2$	88	5.39
3.	9.17	d-Glycero-d-ido-heptose	$C_7H_{14}O_7$	210	23.35
4.	10.78	trans-2-Undecen-1-ol	$C_{11}H_{22}O$	170	8.10
5.	11.03	3-Octyn-2-ol	$C_8H_{14}O$	126	10.19
6.	11.22	3-Nonyn-2-ol	$C_9H_{16}O$	140	6.54
7.	12.52	Imidazole, 2-amino-5-[(2-carboxy)vinyl]-	$C_6H_7N_3O_2$	153	7.58
8.	13.90	Phytol	$C_{20}H_{40}O$	296	15.82
9.	14.56	1-(5-Bicyclo[2.2.1]heptyl)ethylamine	$C_9H_{17}N$	139	1.90
10.	15.16	2-Heptanamine, 5-methyl-	$C_8H_{19}N$	129	1.80
11.	16.19	Thiophene-3-ol, tetrahydro-, 1,1-dioxide	$C_4H_8O_3S$	136	0.63
12.	17.53	Tetrahydro-4H-pyran-4-ol	$C_5H_{10}O_2$	102	1.14
13.	18.89	Methoxyacetic acid, 3-tridecyl ester	$C_{16}H_{32}O_3$	272	1.83
14.	19.57	1,4-Dioxaspiro[4.5]decane, 8-(methylthio)-	$C_9H_{16}O_2S$	188	1.05
15.	20.26	1,3-Butanediol, diacetate	$C_8H_{14}O_4$	174	2.17
16.	21.62	2-t-Butyl-4-methyl-5-oxo-[1,3]dioxolane-4-carboxylic acid	$C_9H_{14}O_5$	202	1.89
17.	22.97	3-Acetoxydodecane	$C_{14}H_{28}O_2$	228	1.25
18.	24.30	2-Aminononadecane	$C_{19}H_{41}N$	283	0.84
19.	26.90	Benzeneethanamine, 2,5-difluoro-á,3,4-trihydroxy-N-methyl-	$C_9H_{11}F_2NO_3$	219	0.63

4.3. Agar Well Diffusion Method:

The Muller Hinton agar medium was prepared dissolving the components in distilled water, and sterilizing by autoclaving at 121°C at 15 psi pressure for 15 mins. The medium was then plated. Organism used for anti-bacterial and anti-sensitivity test under 5 different concentrations are*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*,*Klebsiella pneumonia*, *Escherichia coli*, *Proteus mirabilis.* The result shows that the plant extract with 5 different concentrations like. The zone of inhibition shows that they have the anti-septic and anti-inflammatory activity (Fig. 2).

Fig.2. (a) Control plate **Fig.2. (b) Activity of extract**



5. Discussion:

In spite of tremendous development in the field of synthetic drugs during recent era, they are found that natural herbal plants with various applications have no side effects. In Asia there were tremendous plants with medicinal values. Recent studies suggest that the inflammation and septicaemia are due to the liberation of reactive oxygen species from pathogens invading the inflammation sites. *Elytraria acualis* contains flavanoids, phytol, alkaloids, terpenes, sugar compounds and glycosides. Flavonoids have been shown to possess various biological properties that are related to antioxidant, anti-septic, and anti-inflammatory activities. This targets reactive oxygen species and prostaglandins which are involved in the late phase of acute inflammation and pain perception.

Using the results obtained from GCMS, it was found that the presence of compounds which are active against inflammation and septicaemia. In future studies will be carried out to study the activity of isolated compounds using respective cell line and pharmacological studies of the plant.

6. Conclusion:

In present study it was carried out several tests to evaluate the anti-inflammatory anti septic activity of *Elytraria acualis*. Qualitative and Quantitative phytochemical analysis were done. From the results it was found that our plant species contains many effective compounds like flavonoids, alkaloids, tannin, anthroquinone . Further the sample was analysed using gas chromatography and mass spectrometry (GC-MS). Based on the GC-MS results obtained it was concluded that *Elytraria acualis* have anti-septic and anti-inflammatory activity was confirmed.Further it is planned to do cell line studies using respective animals. There are certain problems associated with use of animals in experimental pharmacological research such as ethical issues and the lack of rationale for their use.

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