IN-VITRO EVALUATION OF EXTRACT OF FABIANA IMBRICATA

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***Abstract*- The study investigated the anti-inflammatory activity of the ethanolic extract of Fabiana imbricate (bad mustard). I have been observed of whole plant of Fabiana imbricate has anti-inflammatory activity. The anti-inflammatory activity of the ethanolic extract of Fabiana imbricate (bad mustard) leaf, stem, root was evaluated using four in vitro- based assays heat induced hemolysis inhibition, Inhibition of albumin denaturation, Anti- proteinase action, preparation of red blood cells (RBCS) suspension. Diclofenac sodium was used as standard, control used as a water. The whole plant was taken too comparable to each other. Results showed that the anti-inflammation activity, the ability of Fabiana imbricate leaf, stem, root extract to inhibit the membrane stabilization in leaf (60µg/ml in 0.17), to inhibit the membrane stabilization in in stem (20µg/ml in 0.4), and to inhibit the membrane stabilization in root (20µg/ml in 0.34), was studied. It was effective in inhibiting the membrane stabilization in leaf (60µg/ml in 0.17), compare to stem and root and control, standard diclofenac sodium (200µg/ml in 0.053). In the present study, results indicate that the ethanolic extracts of Fabiana imbricate possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenyl, the extract fractions serve as free-radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity, and stabilized the Red Blood Cells membrane. In the present study, results indicate that the ethanolic extracts of Fabiana imbricate possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavonoids, tannins, steroids, and phenyl, the extract fractions serve as free-radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity, and stabilized the Red Blood Cells membrane.**

Keywords: **Fabiana imbricate (bad mustard), Anti-inflammatory activity, Ethanolic extracts, and four in vitro-based assays heat induced hemolysis inhibition, Inhibition of albumin denaturation, Anti-proteinase action, preparation of red blood cells (RBCS) suspension.**

I.NTRODUCTION:

Fabiana imbricata (bad mustards) Plants have been used as an excellent source of medicine from the outset, which established a foundation of traditional medicine. Such traditional medicinal plants play a vital role in addressing the global health needs of today and their use will increase in the future[1]. In this study, a number of bad mustard extracts were tested for Anti-inflammatory activity. I was picked Fabiana imbricate Anti-inflammatory activity, based on the plant extracted with ethanolic were collected to extract samples[2]. Fabiana imbricate leaf, stem root (bad mustards) contains many chemical components that are responsible for the achievement of various physiological and therapeutic responses[3]. The extracts of ethanolic then subjected to the various qualitative tests for the detection of Fabiana imbricate constituents like it includes flavanoids, terpenoids, and glycosides, tannins, steroids, and saponins etc. Further, several epidemiological studies also indicated that the incidence of chronic diseases, such as cancer, cardiovascular diseases, and inflammation, is inversely correlated with the consumption of fruits and leafs rich in polyphenols, such as flavonoids[4]. Owing to the side effect of chemical drugs, the use of medicinal plant extract for the treatment of human diseases has greatly increased in the past few decades. The phytochemical in plants act as a medicine; therefore, plants have been used as a source of medicine for thousands of years. I have been taken standard drug sodium citrate, control water, ethanolic extract, with different concentrations, extract used for sampling, according to experimental procedures[5]. All these procedure tested on Anti-inflammatory activity. Therefore, the present study was conducted to determine the anti- inflammatory activity of selected Fabiana imbricate leaf, stem, root using several in vitro bioassays, such as inhibition of albumin denaturation, antiproteinase activity, membrane stabilization, and heat induced hemolysis activity[6]. Inflammation is complex process, which is frequently associated with pain and involves occurrence such as the increase of vascular permeability, increase of protein denaturation and membrane alteration. When cell in the body are damaged by microbes, physical agents or chemical agents, the injury is form of stress[7]. The migration of leukocytes from the venous systems to the site of damage, and the release of cytokines, are known to play a crucial role in the inflammatory response. These chemicals cause widening of blood capillaries (vasodilatation) and the permeability of the capillaries[8]. This will lead to increased blood flow to the injured site.

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 progressive movement of plasma and leukocyte-like constituents from the blood, into the injured tissues locations. Chronic inflammation leads to a progressive shift in the type of cells present at the site of inflammation, and is characterized by simultaneous breakdown and healing of the tissue from the inflammatory process[9]. Non-steroidal anti-inflammatory drugs (NSAID) are commonly used for the management of inflammatory conditions. However, these drugs have several adverse side effects, especially gastric irritation, leading to the formation of gastric ulcers[10]. Therefore, the search for natural

sources and phytochemical with anti-inflammatory activity has greatly increased in recent years. Further, several epidemiological studies also indicated that the incidence of chronic diseases, such as cancer, cardiovascular diseases, and inflammation, is inversely correlated with the consumption of fruits and leafs rich in polyphenols, such as Flavonoids[11]. In the plant kingdom every plant has the potential to produce primary and secondary metabolites which are bioactive in curing many diseases[12]. It includes flavanoids, terpenoids, glycosides, tannins, steroids, and saponins ect. Bioactive compounds from the plants source have the broad spectrum of anti- bacterial, anti-inflammatory activity, anti-fungal, and ant-oxidant activity[13]. Therefore, the present study was conducted to determine the anti-inflammatory activity of selected Fabiana imbricate leaf, stem, root using several in vitro bioassays, such as inhibition of albumin denaturation, antiproteinase activity, membrane stabilization, and heat induced hemolysis activity[14].

MATERIALS AND METHODS:

Materials:

Fabiana imbricate was purchased from the Guntur in Nallapadu. Leaf, stem, root was subjected to pulverization to get coarse powder. It was stored in air tight container further use.

Reagents:

Bovine albumin, 1N HCL, Visible Spectrophotometer, trypsin, tris HCL buffer, casein, perchloric acids, aspirin, saline, NaH_2PO_4, NACL, sodium phosphate buffer pH 7.4 α-amylase enzyme (HiMedia RM 638, Mumbai) acarbose, starch, dinitrosalicylic acid, and chloroform Herbal Science Trust Bangalore. All the other chemicals were procured of analytic grade.

Preparation of crude extract:

30 g of dry power were weighed and transferred to soxhlet apparatus and the extracted with ethanolic at 35°C for 3-4 cycles. The extract was collected and the ethanolic was evaporated after extraction by using rotary evaporator connected to a vacuum pump. The final extract in semi-solid form was dried by placing in desiccators. Until used for the anti-inflammatory bioassays, within one week.

Procedure for in vitro anti-inflammatory activity:

A) Inhibition of albumin denaturation:

The anti-inflammatory activity of Trachyspermum Ammi was studied by using inhibition of albumin denaturation technique which was studied according to followed with minor modification. The reaction mixture was consists of test extracts and 1%aqueous solution of bovine albumin fraction, p H of the reaction mixture was adjusted using small amount of 1N HCL. The sample extracted were incubated at 37 o c for 20 min and then heated to 51 o c for 20min, after cooling the sample the turbidity was measured at 660nm.(UV visible spectrophotometer). The experiment was performed in triplicate. The percentage inhibition of albumin denaturation was calculated as follows.

Percentage inhibition = (abs1 control= abs2 sample) x100/abs control

Whereas,

A1 = absorption of the control sample, and A2 = absorption of the test sample.

(B) Anti-proteinase action:

The test was performed according to the modified method. The reaction mixture (2 ml) was containing 0.06 mg trypsin, 1 ml 20 mm Tris HCl buffer (pH 7.4) and 1 ml test sample of different concentrations (100 - 500 µg/ml). The mixture was incubated at 370 c for 5min and then 1 ml of 0.8% (w/v) casein was added. The mixture was incubated for an additional 20 min. 2 ml of 70% perchloric acid was added to arrest 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 inhibition of proteinase inhibitory activity was calculated.

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

MEMBRANE STABILIZATION

(C) Preparation of Red Blood cells (RBCs) suspension:

The Blood was collected from healthy human volunteer who has not taken any NSAIDs (NonSteroidal Anti-Inflammatory Drugs) for 2 weeks prior to the experiment 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 with isotonic buffer solution (10mm Sodium phosphate buffer pH 7.4). Composition of the buffer solution (g/L) used was NaH_2PO_4, Na_2HPO_4 , and NaH_2PO_4.

(D) Heat Induced Haemolysis:

The reaction mixture (2ml) consisted of 1 ml test sample of different concentrations (100 - 500 µg/ml) and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube. Aspirin was used as a standard drug. All the centrifuge tubes containing reaction mixtures were incubated in water bath at 56 ºC 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. The Percentage inhibition of Haemolysis was calculated as follows:
Percentage inhibition = (Abs control –Abs sample) X 100/ Abs control.

RESULTS AND DISCUSSION

Results and discussion:

Inhibition of albumin denaturation Protein Denaturation is a process in which proteins lose their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. 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 Fabiana Imbricata leaf, Stem, Root extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Maximum inhibition of 71% was observed at 500 µg/ml. Aspirin, a standard anti-inflammation drug showed the maximum inhibition 68% at the concentration of 100 µg/ml compared with control.

Table: 1 IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF (ELEFI)
Membrane Stabilization

S. No	Concentration of Ethanolic Leaf µg/Ml	Absences Value Mean±Sem	Inhibition of Membrane Stabilization
1	CONTROL	0.063	0.063
2	20	0.032	0.4
3	40	0.02	0.6
4	60	0.052	0.17
5	80	0.01	0.84
6	100	0.141	1.23
7	200	0.143	1.26
8	DICLOFENAC SODIUM(200µG/ML	0.053	0.15

Each value represents the mean SD. N=3, experimental group were compared with control ** *p≤0.05, considered significant; ns p≥0.05, non-significant. Ethanolic leaf extracts Fabiana imbricate. (ELEFI)

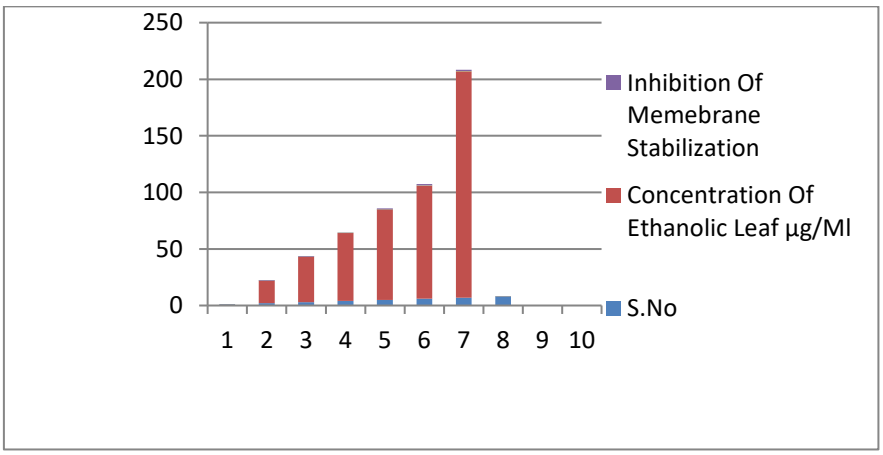

Fig: 1 Concentration of ELEFI

Table: 2 IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF (ELEFI)
Membrane Stabilization

S. No	Concentration of Ethanolic Leaf µg/Ml	Absences Value Mean±Sem	Inhibition of Membrane Stabilization
1	CONTROL	0.063	0.063
2	20	0.033	0.4
3	40	0.016	0.7
4	60	0.243	2.8
5	80	0.423	5.7
6	100	0.431	5.8
7	200	0.62	8.8
8	DICLOFENAC SODIUM(200µG/ML	0.053	0.15

Each value represents the mean SD.N=3, experimental group were compared with control ** *p≤0.05, considered significant; ns p≥0.05, non significant. Ethanolic Stem extracts Fabiana imbricate. (ESEFI)

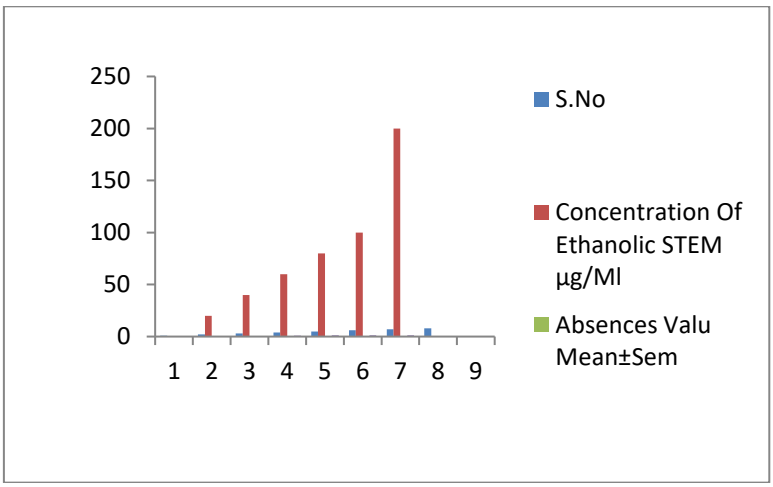

Fig: 2 Concentration of ESEFI

Table: 3 IN-VITRO ANTI-INFLAMMATORY ACTIVITY OF (ELEFI)
Membrane Stabilization

S.No	Concentration Of Ethanolic Leaf µg/Ml	Absences Valu Mean±Sem	Inhibition Of Memebrane Stabilization
1	CONTROL	0.063	0.063
2	20	0.014	0.34
3	40	0.014	0.34

4	60	0.132	1.0
5	80	0.138	1.1
6	100	0.139	1.2
7	200	0.139	1.2
8	DICLOFENAC SODIUM(200µG/ML	0.053	0.15

Each value represents the mean SD.N=3, experimental group were compared with control ** *p≤0.05, considered significant; ns p≥0.05, non significant. Ethanolic Root extracts Fabiana imbricate. (EREFI)

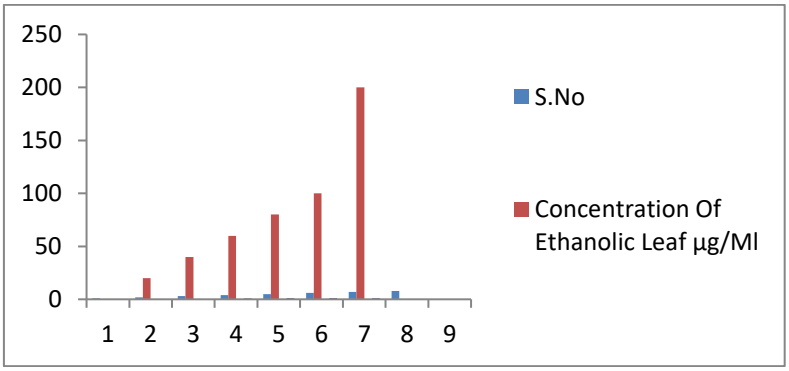

Fig: 3 Concentration of EREFI

CONCLUSION:

ANTI-INFLAMMTORY ACTIVITY:

In the present study, results indicate that the ethanolic extracts of Fabiana imbricata possess anti-inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as alkaloids, flavanoids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or scavenger or acting possibly as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. This study gives on idea that the compound of the plant Fabiana imbricata can be used as lead compound for designing a potent anti-inflammatory drug which can be used for treatment of various diseases such as cancer, neurological disorder, aging and inflammation.

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