PRELIMINARY PHYTOCHEMICAL ANALYSIS, IN VITRO ANTI-ARTHRITIC AND ANTI-INFLAMMATORY ACTIVITY OF AMMANNIA BACCIFEARA

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***Abstract*- The phytochemical screening of the hydroalcoholic extract of Ammannia baccifera. (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, fixed oil, anthocyanin, lecoanthocyanin, emodin, gum, resins, volatile oil. A study has reported that the flavonoids exert membrane stabilizing effect on lysosomes both invitro and invivo in experimental animals. Another report has suggested that tannins and saponins have the ability to bind cations and other biomolecules and are able to stabilize the erythrocyte membrane. HAEAB extract is highly potent on human erythrocyte and thus adequately protecting it against heat and hypotonicity induced lysis. The inhibitory concentration (IC50) of Ammannia baccifera. (Leaf) in HRBC membrane stabilization study is found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml. It showed mild anti-inflammatory activity. The phytochemical analysis showed that the HRBC has flavonoids and tannins. Hence the HRBC membrane stabilizing capacity may be due to the presence of the above mentioned constituents which will prevent the oxidation of haemoglobin and also due to its antioxidant property. The principle involved is the inhibition of protein denaturation. Denaturation of protein was found to be one of the causes of rheumatoid arthritis. In rheumatoid arthritis, the production of autoantigen may be due to protein denaturation which involves the alteration of electrostatic hydrogen, hydrophobic and disulphide bonding. The protein used in this study is bovine serum albumin. Denaturation of protein is carried out by heating. The aim of this activity is to inhibit denaturation and to exhibit protective effect against rheumatoid arthritis. The inhibitory concentration (IC50) of Ammannia baccifera. (Leaf) in Protein denaturation is found to be 17µg/ml in comparison with diclofenac sodium 14µg/ml. It showed moderate anti-arthritic activity. The inhibition of protein denaturation by HAEAB may be due to the presence of phenolic compounds, flavonoids and tannins.**

***Key words:* Preliminary Phytochemical Analysis, In Vitro Anti-Arthritic, Anti-Inflammatory Activity, Ammannia Baccifeara.**

INTRODUCTION:

In ancient times, traditional systems of medicine were the fundamental source of herbal medications [1]. A majority of the population is dependent the on use of various species of herbal remedies to treat health problems [2] because of the insufficient availability of modern medicine, particularly in rural areas [3].

Rheumatoid arthritis (RA) is an autoimmune disorder, which can result in chronic inflammation in the synovial membrane and also cause pain in small and large joints, as well as the destruction of cartilage and bone [4]. The characteristic features of RA are joint pain, immobility and malformation [5]. The management of RA is mainly achieved through the use of nonsteroidal anti-inflammatory drugs (NSAIDS) such as indomethacin, ibuprofen, aspirin, and naproxen, but these only manage it for a short time duration [6]. The arthritic and anti-inflammatory action of NSAID is attributed to it cyclooxygenase (COX-1 and 2) inhibition, as well as its inhibition of the pro cytokinins (IL1, IL-6 TNF-alpha, etc.), curing arthritic disease [7]. Some NSAID has a short duration of action and can also produce some negative side effects in the epigastric region [7]. When inflammation occurs, macrophage cells are released into the injured tissue area, which can cause life-threatening diseases like Alzheimer's, arthritis, cancer, allergies, and atherosclerosis, as well as autoimmune diseases [1]. Inflammation causes the vasodilation of capillaries

and increases the blood flow to the injured region [8].
Oxidation causes chemical and physiological changes in the biological system in living organisms and organic substances, which can be oxidised as a result of various physicochemical processes such as exposure to heat, light or any other oxidising agents [9]. Reactive oxygen species (ROS) are produced from the oxidised bioactive constituents, as a result of the direct exposure of highly reactive molecules that are abundant in living tissue to the atmosphere or during aerobic metabolism [10]. ROS circulating in the blood stream affects the metabolic process because it reacts with the free electron molecules that are present in living systems, which can lead to various life-threating diseases like ischemia, respiratory distress, arthritis, cancer, and aging, and can also damage various vital organs. Herbal medicines have numerous bioactive phytoconstituents, and have the ability to quench free radical oxygen species; this contributes beneficial effects towards life-threatening diseases in our body [11]. *Ammonia baccifera* Linn (Lythraceae) is a glabrous, erect branching herb, found as weed in rice-fields and marshy localities throughout India. Leaves sessile, quadrangular, young stem and lower stem tinged, axillary inflorescence, sessile flowers, red in colour and globose fruit capsule.
Ammannia baccifera is widely used in traditional Chinese/Indian herbal formulations for treating human female infertility, gastroenteropathy, spinal disease, hemorrhoids, urethritis, common cold, abscess, sore, itching and other skin diseases.
It has been reported to possess anticancer, antirheumatic, antidiuretic, antipyretic, antisteroidogenic, antimicrobial, rubefacient and antiurolithic activities.

MATERIALS AND METHODS

PLANT COLLECTION & AUTHENTIFICATION

Fresh leaf of *Ammannia baccifera* were collected from Chittor, AP, during the month of August- 2023 and was authenticated.

QUALITATIVE AND QUANTITATIVE ANALYSIS

The hydroalcoholic extract was subjected to qualitative and quantitative analysis. Qualitative analysis includes phytochemical screening of secondary metabolites such as flavonoids, carbohydrates, alkaloids, glycosides, sterols, tannin, protein, aminoacids, carotenoids, volatile oil, quinone, terpenoids, phenolic content and Thin layer chromatography of the extract were determined. Quantitative analysis includes estimation of total tannin, total gallic acid, total flavonoid contents in terms of total tannic acid equivalent, total gallic acid equivalent, total flavonoids equivalent (rutin) and total carotenoid and total chlorophyll content and extract were determined.

QUALITATIVE ANALYSIS

PRELIMINARY PHYTOCHEMICAL SCREENING

Hydroalcoholic extract of *Ammannia baccifera*. (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of flavonoids, phenolic compounds, alkaloids, glycosides, carbohydrates, carotenoids, proteins, tannin, aminoacids, sterols as per Harborne 1998.

PHARMACOLOGICAL STUDIES

ANTI-INFLAMMATORY ACTIVITY

Medicinal and culinary herbs are rich sources of anti-inflammatory compounds such as flavonoids. Pharmaceutical drugs are built upon a single molecule while herbal remedies contain different active ingredients. One of the wide spread complaint against modern medicines is its side effect that can be attributed to a single biochemical pathway that is triggered by the molecule of interest. On the contrary herbal medicines mediate multifaceted biochemical attack on inflammation due to the diversity and synergy of the anti-inflammatory compounds. Inflammation is a protective response by our immune system against organisms which cause cell injury (e.g., microbes, toxins) and deals with the consequences of such injury. It may be acute or chronic, depending up on the nature of stimulus and the effectiveness of initial reaction in eliminating the stimulus or the damaged tissues. The main components of inflammation are a vascular reaction and a cellular response, both are activated by mediators that are derived from plasma proteins and various cells. The outcome of acute inflammation is either elimination of the noxious stimulus followed by decline of the reaction and repair of the damaged tissue, or persistent injury resulting in chronic inflammation.

Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain. Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hey fever, ischemic heart diseases and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, syphilis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases, auto- immune

diseases etc.
Anti-inflammatory drugs like NSAIDs used to reduce the swelling and pain of inflammation. But these agents carry the risk of gastro-intestinal toxicity, cardiovascular and other toxicity for prolonged use. For these reason, there is a need for ant-inflammatory drugs
having less severe side effects to use for chronic inflammatory disease as well. Therefore, in recent time, more interest is shown in alternative and natural drugs for treatment of various diseases, but there is a lack of proper scientific evidence.
Leukocytes, the key players of inflammatory response, can eliminate microbes and dead cells by phagocytosis, followed by their destruction in phagolysosomes. Destruction is caused by free radicals generated in activated leukocytes (neutrophils and monocytes) and lysosomal enzymes. Enzymes and reactive oxygen species may be released into the extracellular environment where it acts as mediators of inflammation. Such mediators are mainly arachidonic acid metabolites, generated through Cyclooxygenase and Lipoxygenase pathways. Most of the anti-inflammatory drugs are targeted on these pathways.
In a different approach, rather than blocking a particular mediator or its pathway, preventing the release of inflammatory mediators could be considered as a better option. The possibility of this approach is revealed in this research by studying the ability of the plant extract to prevent the lysosomal membrane destruction. An effective way to study this activity in vitro is to study the HRBC membrane stabilization activity of the plant extract. Lysosomal membrane and RBC membrane are similar in structure apart from the fact that luminal surface of the lysosomal membrane contains a glycoprotein coat which protects the membrane from digestion by lysosomal acid hydrolases. This method has been used in most preliminary anti-inflammatory screening procedures. Plants produce different bioactive compounds using secondary metabolic pathways in response to specific environmental stimuli such as herbivore-induced damage, pathogen attacks, or nutrient depravation. These secondary metabolites can be unique to specific species or genera and perform a host of general, protective roles including anti-inflammatory and antioxidant activities.
Ammannia baccifera. commonly known as ‘Benghal dayflower’ or ‘tropical spiderwort’ is a perennial herb and its young leaves are eaten as vegetables. It is used as a folk medicine for the variety of ailments in the Indian subcontinent. It has antibacterial, sedative, anxiolytic, analgesic and anticancer properties and used against diuretic, febrifuge, inflammatory and leprosy problems. The phytochemical studies of *Ammannia baccifera.* revealed the presence of flavonoids and phenolic compounds. The current study focuses on the evaluation of in vitro anti-inflammatory property and phytochemical nature of the leaf extracts of *Ammannia baccifera.*

INVITRO ANTI-INFLAMMATORY ACTIVITY SCREENING BY MEMBRANE STABILIZATION STUDY

Principle

The method of Sadique *et al.*, (1989) and modified by Oyedapo and Famurewa (1995) and Oyedapo *et al.*, (2012) was employed in the membrane stabilizing activity assay. When RBCs are subjected to heat and treatment with hyposaline they release haemoglobin which has a maximum absorbance at about 560 nm. The capacity of the extract to reduce hyposaline and heat induced lysis is basis of the assay.

Instrument

Shimadzu UV visible spectrophotometer, Model 1800

Materials required

70% Hydroalcoholic extract of *Ammannia baccifera.*
0.2 M Sodium phosphate buffer (p^H 7.4)
0.36% w/v Hyposaline
10% v/v HRBC suspension in isosaline

Preparation of HRBC suspension in isosaline

The human erythrocytes suspension was used for the *in-vitro* membrane stabilization assay. Blood was collected from the healthy volunteers who had not consumed any NSAIDs for two weeks prior to the experiment. The blood was mixed with equal volume of Alsever’s solution (2% dextrose, 8.0% sodium citrate, 0.5% citric acid, 0.42% sodium chloride) and centrifuged at 3000 rpm. The packed cells were washed with isosaline and a 10% v/v erythrocyte suspension in isosaline was prepared.

Procedure

The assay mixture consist of 2 ml of hyposaline and 1 ml of phosphate buffer and varying volumes (0.1 to 0.5 ml) of

HAEAB extract at different concentration (10, 20, 30, 40, 50 µg/ml) and 0.5 ml HRBC suspension in isosaline, then the final volume were made upto 4.5 ml with isosaline. The control was prepared as mentioned above without the test extract, while drug control was also prepared similarly but without HRBC suspension. The reaction mixture was incubated at 56º C for 30 min in a water bath, then the tube was cooled under running water. Then the absorbance of the released haemoglobin was measured at 560 nm. Diclofenac sodium was used as a reference standard.

RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by joint swelling, synovial inflammation and cartilage destruction and commonly lead to significant disability. According to WHO, 0.3-1% of the world population is affected from rheumatoid arthritis (RA) and among them females are three times more prone to the disease as compared to males. It caused by number of proinflammatory molecules released by macrophages including reactive oxygen species and ecosanoids such as prostaglandins, leukotrines and cytokines. The regulation of these mediators secreted by macrophages and other immune cells and modulation of arachidonic acid metabolism by inhibiting enzymes like cox and lox are the potential target for chronic inflammatory conditions. Eventhough various categories like immunosuppressants, NSAIDs, steroidal anti-inflammatory drugs are being used till now, the potential side effects give a limitation for their use. Now it is a growing concern all over for the development of new safe, potent, less toxic antiarthritic drug . Hence, there is a need to explore for more naturally available alternatives, so that their therapeutic values can be assessed and expanded.

Plants are one of the most important sources of medicines. India is known as the “Emporium of Medicinal plants” due to availability of several thousands of medicinal plants in the different bioclimatic zones anti-inflammatory diseases including rheumatoid arthritis are still one of the main health problems of the world’s population. The use of natural remedies for the treatment of inflammatory and painful conditions has a long history, starting with Ayurvedic treatment, and extending to the European and other systems of traditional medicines. Plant drugs are known to play a vital role in management of inflammatory diseases.

INVITRO ANTIARTHRITIC ACTIVITY BY PROTEIN DENATURATION METHOD

Rheumatoid arthritis is an autoimmune disorder. One among the cause for the disease is due to the denaturation of the protein. Antiarthritic activity was studied by inhibition of protein denaturation method.

Materials required

70% Hydroalcoholic extract of *Ammannia baccifera.*
Diclofenac sodium
Bovine serum albumin (5% w/v aqueous solution) Phosphate buffer (P^H 6.3)

Instrument

Shimadzu UV visible spectrophotometer, Model 1800

EXPERIMENTAL PROTOCOL

The following four solutions were prepared

1. Test solution (0.5 ml)

The test solution consists of 0.45 ml bovine serum albumin (5% w/v aqueous solution) and 0.05 ml of HAEAB (10, 20, 30, 40, and 50 µg/ml concentration).

2. Test control solution (0.5 ml)

The test control solution consists of 0.45 ml bovine serum albumin and 0.05 ml distilled water.

3. Product control (0.5 ml)

The product control consists of 0.45 ml of distilled water and 0.05 ml of HAEAB (10, 20, 30, 40, and 50 µg/ml concentration).

4. Standard solution

Standard solution consists of 0.45 ml of bovine serum albumin and 0.05 ml of Diclofenac sodium solution.

All the above test samples was adjusted to p^H 6.3 using a small amount of 1N hydrochloric acid. They were incubated at 37º C for 20 minutes and heated at 57º C for 3 minutes. Allow to cool and about 2.5 ml of phosphate buffer (p^H 6.3) was added to all the above solution. The absorbance was measured using UV spectrophotometer at 416 nm.

RESULTS AND DISCUSSION

Preliminary phytochemical screening of Aqueous and Hydro – Alcoholic extract of

***Ammannia baccifera*. (Leaf)**

Hydroalcoholic extract of *Ammannia baccifera*. (Leaf) was subjected to qualitative chemical analysis. The various chemical tests were performed on this extract and aqueous extract for the identification of phytochemicals, secondary metabolites and the results are displayed in **Table: 1**

Table: 1 Preliminary phytochemical screening of Aqueous and Hydro – Alcoholic extract of *Ammannia baccifera*. (Leaf)

S.NO	Test	Hydroalcoholic extract of *Ammannia baccifera* *L*. (Leaf)	Aqueous extract of *Ammannia baccifera* *L*. (Leaf)
1	**Alkaloids**		
	Mayer' Test	Positive	Positive
	Dragendorff's Reagent	Negative	Negative
	Hager's Reagent	Positive	Positive
	Wagner's Reagent	Positive	Positive
2	**Carbohydrates**		
	Benedict's Test	Positive	Positive
	Fehling's Test	Positive	Positive
	Molisch's Test	Positive	Negative
3	**Anthraquinone Glycoside**		
	Borntrager's Test	Negative	Negative
4	**Cardiac Glycosides**		
	Keller killiani Test	Negative	Negative
	Legal Test	Negative	Negative
5	**Sterols**		
	Salkowski's Test	Positive	Positive
	Libbermann-Burchard's Test	Negative	Negative
6	**Saponins**	Positive	Positive

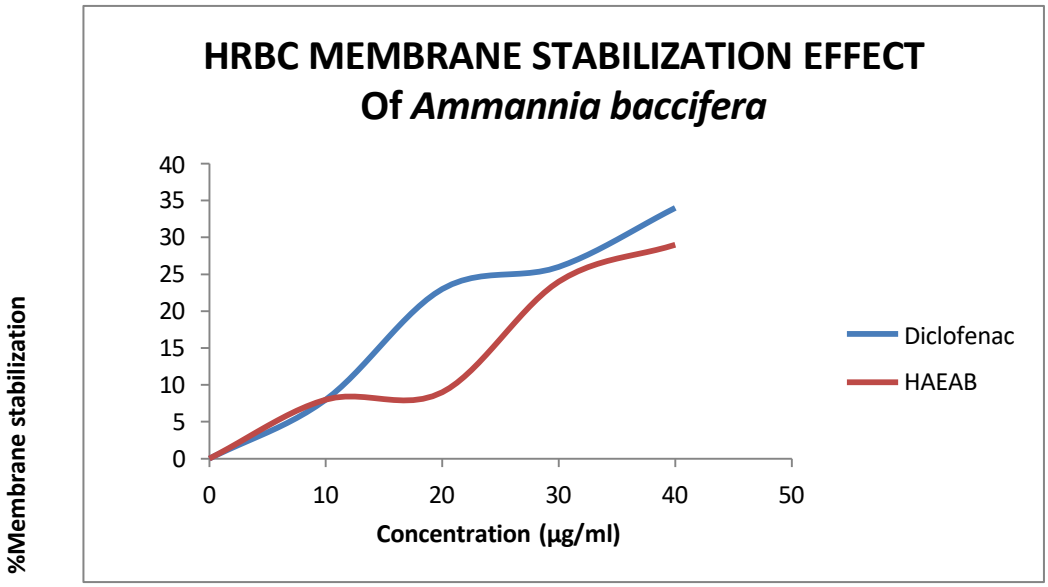
7	**Tannins and Phenolic compounds**		
	Folinciocalteu's phenol Reagent	Positive	Positive
	$Fecl_3$ Test	Positive	Positive
8	**Flavonoids**		
	Shinoda Test	Positive	Positive
	Lead Acetate Test	Positive	Positive
	Acid Test	Positive	Positive
	Alkali Test	Positive	Positive
9	**Protein and Free Amino Acids**		
	Biuret Test	Positive	Negative
	Ninhydrin Test	Positive	Positive
	Sulphur containing Amino Acid	Positive	Positive
10	Mucilage	Positive	Positive
11	Quinone	Positive	Positive
12	Phlobatannins	Positive	Positive
13	Terpenoids	Positive	Negative
14	Betacyanins	Positive	Negative
15	Emodin	Negative	Positive
16	Fixed oil	Negative	Negative
17	Gum	Negative	Negative
18	Anthocyanins	Negative	Negative
19	Lecoanthocyanins	Negative	Negative
20	Resins	Negative	Negative
21	Volatile oil	Negative	Negative

The phytochemical screening of the hydroalcoholic extract (70%) of *Ammannia baccifera*. (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, fixed oil, anthocyanin, lecoanthocyanin, volatile oil,emodin, gum, resins,. The aqueous extract of *Ammannia baccifera.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, mucilage, emodin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, terpenoids, fixed oil, betacyanin, gum, anthocyanin, lecoanthocyanin, resins, volatile oil.

ANTI-INFLAMMATORY ACTIVITY

Hydroalcoholic extract of *Ammannia baccifera.* was subjected to Anti- Inflammatory activity by Membrane stabilization method.

FIGURE:1 PERCENTAGE OF MEMBRANE STABILIZATION BY DICLOFENAC SODIUM AND HAEAB



The inhibitory concentration (IC_{50}) of *Ammannia baccifera.* (Leaf) in HRBC membrane stabilization study is found to be 69µg/mLin comparison with diclofenac sodium57µg/mL.

Table:2 PERCENTAGE OF MEMBRANE STABILIZATION BY DICLOFENAC SODIUM AND HAEAB

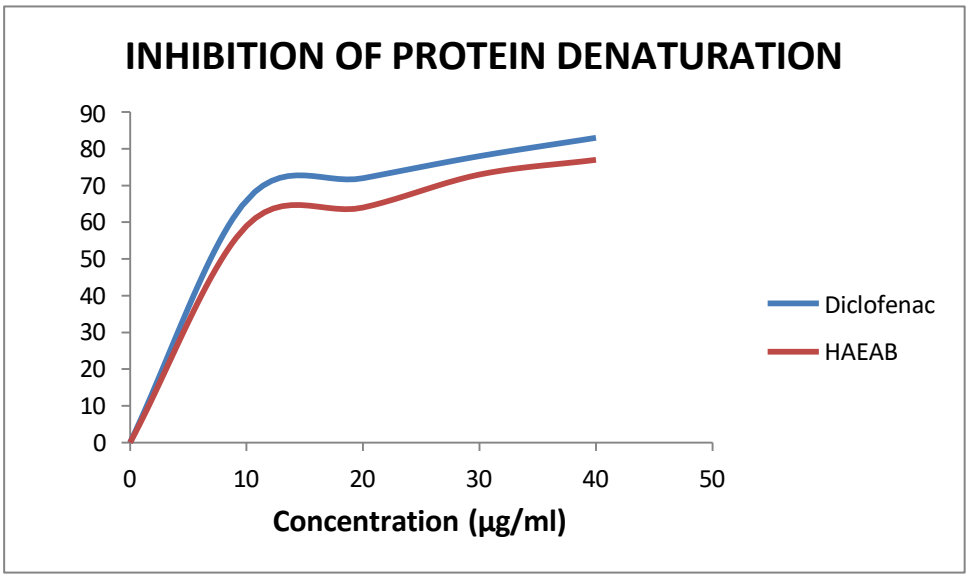
S.NO	Concentration (µg/ml)	Percentage membrane stabilization of Diclofenac*	Percentage membrane stabilization of HAEAB*
1	10	8.14 ± 0.0088	7.7 ± 0.0577
2	20	22.75 ± 0.0088	9 ± 0.0577
3	30	25.9 ± 0.0088	23.5 ± 0.1453

4	40	34.4 ± 0.3333	29.3 ± 0.0882
	IC_{50}	57 µg/mL	69 µg/mL

*Mean of three readings ± SEM
HAEAB showed mild anti-inflammatory activity.

ANTI-ARTHRITIC ACTIVITY
Hydroalcoholic extract of *Ammannia baccifera*. was subjected to Anti-Arthritic activity by Protein denaturation method.

Figure: 2 Effect of HAEAB and Diclofenac Sodium on Inhibition of Protein Denaturation



The inhibitory concentration (IC_{50}) of *Ammannia baccifera*. (Leaf) in Protein denaturation is found to be 17µg/mL in comparison with diclofenac sodium 14µg/mL.

Table: 3 Effect Of HAEAB and Diclofenac Sodium on Inhibition of Protein Denaturation

Sl. NO	**Concentration in µg/ml**	**% Inhibition**	
		Diclofenac	**HAEAB**
1	10	65.6 ± 0.3464	59.4 ± 0.2309
2	20	71.9 ± 0.5774	64.1 ± 0.0882
3	30	78.1 ± 0.0882	73.4 ± 0.2309
4	40	82.8 ± 0.5774	76.6 ± 0.1732
	IC_{50}	14 µg/mL	17 µg/mL

*Mean of three readings ± SEM
HAEAB showed moderate anti-arthritic activity.

SUMMARY

The phytochemical screening of the hydroalcoholic extract (70%) of *Ammannia baccifera*. (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. It shows the absence of anthraquinone glycosides, cardiac glycoside, fixed oil, anthocyanin, lecoanthocyanin, emodin, gum, resins, volatile oil. The aqueous extract of *Ammannia baccifera*. (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, mucilage, emodin, quinone, phlobatannins, It shows the absence of anthraquinone glycosides, cardiac glycoside, terpenoids, fixed oil, betacyanin, gum, anthocyanin, lecoanthocyanin, resins, volatile oil.

Invitro membrane stabilization study

Lysosomes are intracellular particles which contain most of the lytic and digestive enzymes of the tissue. The rupture of the lysosomes results in injury or death to surrounding tissues and also acute inflammation. The membranes of lysosomes and erythrocytes are destroyed by similar agents; hence a test was developed to measure the ability of compounds to stabilize erythrocyte membrane to heat hemolysis. RBC when exposed to various injurious substances such as methyl salicylate, phenyl hydrazine, and hypotonic medium or over heat will cause lysis of membrane accompanied by haemolysis and oxidation of haemoglobin. RBC membranes are easily susceptible to free radical mediated lipid peroxidation by breakdown of biomolecules. Due to it has rich source of iron and high oxygen partial pressure. RBC membranes are similar to lysosomal cells. All NSAIDs inhibited hemolysis while other type of compounds had no effect. Hence prevention of hypotonic and heat mediated RBC membrane lysis taken as measure of anti-inflammatory activity of drugs.

A study has reported that the flavonoids exert membrane stabilizing effect on lysosomes both invitro and invivo in experimental animals. Another report has suggested that tannins and saponins have the ability to bind cations and other biomolecules and are able to stabilize the erythrocyte membrane. HAEAB extract is highly potent on human erythrocyte and thus adequately protecting it against heat and hypotonicity induced lysis. The inhibitory concentration (IC_{50}) of *Ammannia baccifera*. (Leaf) in HRBC membrane stabilization study is found to be 69µg/ml in comparison with diclofenac sodium 57µg/ml. It showed mild anti-inflammatory activity.

The phytochemical analysis showed that the HRBC has flavonoids and tannins. Hence the HRBC membrane stabilizing capacity may be due to the presence of the above mentioned constituents which will prevent the oxidation of haemoglobin and also due to its antioxidant property.

Invitro antiarthritic activity by protein denaturation method

The principle involved is the inhibition of protein denaturation. Denaturation of protein was found to be one of the causes of rheumatoid arthritis. In rheumatoid arthritis, the production of autoantigen may be due to protein denaturation which involves the alteration of electrostatic hydrogen, hydrophobic and disulphide bonding.

The protein used in this study is bovine serum albumin. Denaturation of protein is carried out by heating. The aim of this activity is to inhibit denaturation and to exhibit protective effect against rheumatoid arthritis. The inhibitory concentration (IC_{50}) of *Ammannia baccifera*. (Leaf) in Protein denaturation is found to be 17µg/ml in comparison with diclofenac sodium 14µg/ml. It showed moderate anti-arthritic activity. The inhibition of protein denaturation by HAEAB may be due to the presence of phenolic compounds, flavonoids and tannins.

CONCLUSION

- Quantitative estimation helps to identify the gallic acid, tannic acid, and flavonoids equivalents present in the hydroalcoholic extract of *Ammannia baccifera*.
- The phytochemical screening of the hydroalcoholic extract (70%) of *Ammannia baccifera.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, terpenoids, mucilage, betacyanin, quinone, phlobatannins, carotenoids. The aqueous extract of *Ammannia baccifera.* (Leaf) powder revealed the presence of alkaloids, carbohydrates, sterols, saponins, tannins and phenolic compound, flavonoids, protein and free aminoacid, mucilage, emodin, quinone, phlobatannins, carotenoids.
- Pharmacological screening potentiates the biological invitro anti-inflammatory effect, invitro anti-arthritic effect.
- At the site of inflammation, HAEAB may possibly inhibit the release of lysosomal content of neutrophils (bactericidal enzymes and proteinases) which upon extracellular release cause further tissue inflammation and damage (Chou, 1997). In the present study, results indicate that the HAEAB possesses significant anti-inflammatory properties which may be due to the strong occurrence of polyphenolic compounds such as flavonoids, tannins and phenols.
- HAEAB showed significant antiarthritic activity by inhibition of protein denaturation. Rheumatoid arthritis

(RA) being a common inflammatory disease affects about 1% of the adult population worldwide. It occurs in immunogenetically predisposed individuals. Protein denaturation was found to be one of the causes of RA. HAEAB has shown significant anti-arthritic activity and the phenolic constituent may be responsible for this activity.

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