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Preliminary Phytochemical Screening And Evaluation Of Invitro Anti-Inflammatory Activity Of Ethanolic Flower Extract Of *Tabernaemontana Divaricata*

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Abstract

There are numerous medicinal plants in india. With the development of science and technology, the general people has been using this medicinal plant extensively in an effort to come back health issues like pain and inflammation . The vital significance of secondary metabolites of plants as therapeutic raw materials has been further classified by discovery of numerous new pharmaceutical molecules derived from natural sources .The plant *Tabernaemontana Divaricata* also known as pinwheel flower is a member of the Apocynaceae .The current study examines preliminary phytochemical and anti-inflammatory screening of *Tabernaemontana Divaricata*.The flower has anti-inflammatory activity alkaloids , flavonoids , tannins, glycosides, terpenoids. To identify the components in the ethanolic floral extract by performing HRBC Membrane stabilization method &Inhibition of protein denaturation method.The anti-inflammatory properties of crude ethanolic extract of *Tabernaemontana Divaricata* were examined.

Index Terms

Crape jasmine, Pinwheel flower, Nandi vardhana, phytochemical screening, ethanolic floral extract ,Anti - inflammatory activity.

Introduction

Plants accommodate many pharmaceutical Agent that save human life mostly the essential needs. In many countries for instance India and China, plants have formed point of departure of traditional medicine (Singh et al 2006). In developing countries, most of the paramount sources of medicines are plants, which mostly depend on plant found medicines for healthcare purposes (Ramesh & Okigbo et al2008). Over two billion people throughout the world deliberately depend on plants that have medicinal role (Smith-Hall et al 2012). In developing countries, since 1990's, traditional medicine has gained popularity according to WHO survey. Plants act as predominant source of nutrition for producing human's physiological activity (Olowa

& Nuneza et al 2013). In drug synthesis and development, plants are endorsed for their therapeutic values (Ramawat & Merillon 2008).

In case of therapeutic efficacy, pharmacologically active compounds existing in medicinal plants are useful (Salim et al 2005). The original or semi synthetic forms of secondary metabolites for instance resins,tannins, alkaloid and essential oils are mostly preferred (Olowa & Nuneza 2013; Rasool Hassan et al 2012). Through, having significant therapeutic potential, they produce adverse effects when consumed in large amounts due unknown determined substance (Melanie et al1997). Herbal procured remedy is strong and bottomless appraisal on the pharmacological activities (Firenzuoli & Gori 2007).Human life is highly benefited regarding their health and also prominent firearm in brawling against bacterial infections by using Antibiotics. Nevertheless, from past few decades, due to emergence of drug resistance against bacteria and production of toxic reaction these become less effective against particular ailments. So it is important to scrutinize brand new drug with lower resistance. Both in prevention and treatment of human affliction, drug obtained from natural source plays a paramount role. Traditional medicine acts as one of the primary healthcare systems in most of developing countries. Herbs are extensively utilized in traditional medicine and their therapeutic capabilities are skilfully registered. Newly developed drugs between 1981 and 2002 are extracted from natural products which are prosperous and scientifically in cancer and various infections afflictions. Discovery outlay of many active novel chemicals entities is declined in the recent days. New source of antimicrobial agents are produced by hardback mechanisms from higher plants. Newly developed drugs between 1981 and 2002 are extracted from natural products which are prosperous and scientifically in cancer and various infections afflictions. Discovery outlay of many active novel chemicals entities is declined in the recent days. New source of antimicrobial agents are produced by hardback mechanisms from higher plants.

Figure-1. *Tabernaemonatana Divaricata*

PHYTOCHEMISTRY

Plant phytoconstituents can be used for a variety of medicinal purposes. *Tabernaemontana divaricata* contains various chemical constituents such as alkaloids, flavonoids, tannins, glycosides and terpenoids. Alkaloids like ibogamine, coronaridine, and voacangine, ibogaine& Flavonoids like quercetin and kaempferol

PLANT COLLECTION AND AUTHENTICATION

Tabernaemontana divaricata flowers are acquired from surroundings village, G.Kothapalli, Dr.G. Jyothirmaiyee, Department of Botany, S.R.V.B.S.J.B.Maharani College, Peddhapuram,authenticated and taxonomically recognised the flower material.

EXPERIMENTAL METHODOLOGY

Preparation of powdered flower for extraction:

In order to prevent chemical contents from degrading, the *Tabarnaemontana Divaricata* flowers that have been harvested are cleaned and dried at room temperature. Recently convened flowers of *Tabernaemontana divaricata* were collected at their fully mature form, then dehydrated below shadiness for nearly 15 days and pulverized powder using mechanical grinder. Then granulated one is squashed using Ethanol for about

3 days and then exposed about 3hrs for hot percolation. The solution is then allowed for filtration followed by concentration. The concentrated liquid is subjected to distillation at 80°c. The appearance of solvent is taken a back the ending of extraction. The distillate is then shifted into earlier used china dish and then dehumidified into dark paste using water bath at 50°c to form Ethanolic extract. Concentrated product then exposed to drying using desiccators using anhydrous CaCl2.After being ground into a coarse powder, these leaves are used in the study.

PRELIMINARY PHYTOCHEMICAL SCREENING:

For quantitative determination of phytochemical constituents the following chemical tests are carried out.

TESTS FOR ALKALOIDS:

1.Dragendroffs test : (solution of potassium bismuth iodide)

1 ml of the distillate added to equal amount of Dragendroff"s reagent a reddish-brown precipitate shows the alkaloids presence.

2.Mayer's test : (potassium magnesium iodide)

1ml of distillate added to equal amount of Mayer's reagent cream precipitate shows the presence of alkaloid.

3.Hager's test : (picric acid saturated solution)

1ml of distillate add little amount of Hager's reagent reddish brown precipitate shows the presence of alkaloids.

TESTS FOR CARBOHYDRATES

1.Molisch test:

Sodium extract is mixed with Molisch reagent and concentrated sulphuric acid is added through the sideways. Purple colour formed at the ring junction of two liquids shows the presence of carbohydrates.

2.Benedicts test: Solution of extract is mixed with Benedict's reagent and allow for boiling on water bath. Observation of reddish-brown precipitate indicates the existence of carbohydrates.

3.Fehling's test:

Solution of extract is mixed with little amount of Fehling's A&B solutions and allowed for boiling Brick red colour indicates the presence of carbohydrates.

TESTS FOR FLAVONOIDS

1.Lead acetate test:

Extract solution is added in little amount to 10% lead acetate which results in yellow precipitate formation.

2.Zn-Hcl reduction test:

Extract solution is added to zinc and concentrated hydrochloric acid.The reaction mixture shows magenta colour which is due to the presence of flavonoids.

3.NaOH test:

Solution of extract is added to NaOH solution indicates yellow colour which is highly intense in nature. Later existence of colour solution by adding diluted HCl which shows existence of flavonoids.

TESTS FOR GLYCOSIDES:

General tests:

To the extract 5 ml dil. H_2SO_4 is added. Warm and filter. Neutralize the acid with 5% NaOH. Add 0.1 ml of Fehling's a and b solution until it becomes alkaline and heat
for 2 min . Red precipitate formed shows the existence of glycosides.

Modified born taggers test:

Distillate is hydrolyzed with conc. HCl for hours using water bath and then exposed to 2 ml of hydrolyzed extract dil.H_2So_4 is added. Then boiled and filtered. Cool and add equal amount of petroleum ether. Shake and separate organic layer and to it add equal volume of ammonia solution. When organic layer turns pink shows presence of glycosides.

Legal's test:

Extract is hydrolyzed with Conc.Hcl for hours using water bath and exposed to legal's test. 2ml of few drops of hydrolyzed extract of 10%NaOH and freshly prepared sodium nitroprusside is added. Blue colour indicates the presence of glycosides.

TESTS FOR PROTIEINS

Xanthoproteic test:

To 3ml of extract solution add 1ml of con.h2so4. Formation of white precipitate shows the existence of colour proteins

Millions test:

To 3ml of extract solution add 5 ml of millions reagent. A white precipitate is formed when warmed precipitate turns red dissolves by giving red colour to the solution.

Biuret test:

Solution of extract is treated with 4% NaO H solution and two drops of 1%CuSO4 solution and observed for the formation violet /pink colour.

TEST FOR SAPONINS

Foam and froth test:

Test solution was diluted with water and shaked vigorously and examined for the formation of froth it is stable for 15 minutes for a positive result.

TESTS FOR TANNINS:

Ferric chloride test:

To few ml of the extract add1% FeCl3 solution gives blue, green, brownish green colour if tannins are present.

TESTS FOR TRITERPINOIDS:

Salkowski test:

Test extract was shaked with chloroform layer sulphuric acid was added slowly by walls of test tube. Red colour indicates the presence of Triterpenoids.

Libermann-Buchards test:

2mg of dried extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the sides of the test tube. Formation of violet colour ring indicates the presence of Triterpenoids.

ANTI-INFLAMMATORY ACTIVITY:

Procedure

Preparation of reference drug

NSAID (Ibuprofen) were used as reference drug. Ibuprofen was crushed into fine powder.
About 0.2g of Ibuprofen drug powder was measured using a digital analytical balance (Adam PW 254)
And was added to 20.0ml of distilled water, respectively. The solution was mixed well using a vortex.
Serial dilution from 1000µg/ml to 10µg/ml was performed for *Tabernaemontana divaricata* extract and for reference drug. All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8ml of phosphate-buffered saline (Ph6.4) and 2ml of egg albumin (from fresh hen's egg). Then 2ml of *Tabernaemontana divaricata* flower extract from each different concentration were mixed gently with reaction mixtures. Similar procedure was used for reference drug and these are used as positive control. In addition, distilled water was used as negative control.

1. HRBC Membrane stabilization method:

Procedure

The blood was collected from healthy human volunteer (who had not taken any NSAIDS for 2 weeks prior to the experiment).Then it was Mixed with equal volume of Alsever solution (2% dextrose, 0.8% sodium citrate,0.5% citric acid and 0.42% NaCl) and centrifuged at 3,000 rpm.The packed cells were washed with Isosaline and a 10% suspension was made.Various concentrations of extracts were prepared (100 and 200 µg/ml) using distilled water and To each concentration 1 ml of phosphate buffer, 2 ml hyposaline and 0.5 ml of HRBC suspension were added.It was incubated at 37˚C for 30 min and centrifuged at 3,000 rpm for 20 min. and the haemoglobin content of the supernatant solution was estimated on UV spectrophotometer at 560nm.Ibuprofen (100 and 200 g/ml) was used as reference standard and a control was prepared by omitting the extracts

Fig . HRBC Membrane stabilization Method

2. INHIBITION OF PROTEIN DENATURATION METHOD :

Procedure

Reaction mixtures were incubated in a water bath at 37˚c±2˚c for 15 -20 min, and later, It was heated at 70˚c at which the reaction mixture was maintained for 5min.Then, the reaction mixture was allowed to cool down at room temperature for 15min.Absorbance of reaction mixture before and after denaturation was measured for each concentration(1000µg/ml,100µg/ml, and 10µg/ml) at 680nm using a colorimeter Each test was repeated thrice and the mean absorbance was recorded .The percentage of inhibition of protein was

determined on a percentage basis with respect to control using the following formula.

Fig . Inhibition of Protein denaturation method

Percentage of inhibition = (Absorbance of control-absorbance of test)/absorbance control×100

RESULTS

Phytochemical analysis:

Various chemical tests are carried out and tabulated below. Fundamental phytochemical tests confessed the existence of alkaloids, flavonoids, tannins, Triterpenoids and proteins.

COMPOUNDS	CHEMICAL TEST	RESULTS
Alkaloids	Dragendroff's	+ve
	Hager's test	+ve
Glycosides	General test	-ve
Flavonoids	Lead acetate test	+ve
	Zinc hydrochloride test	-ve
	NaOH test	+ve
Saponins	Froth formation test	+ve
Triterpenoids	Salkowski test	-ve
Tannins	Ferric chloride test	+ve
Carbohydrates	Molisch's test	+ve
	Benedict's test	+ve
	Fehling's test	+ve
Proteins	Xanthoproteic test	+ve
	Millons test	+ve
	Biuret test	-ve

Table no 01: Results of Phytochemical Analysis of Ethanolic Flower Extract of *T.divaricata*

Invitro anti-inflammatory activity :

HRBC METHOD:-

Type of extract	Concentration µg/ml	Absorbance	%Inhibitionof denaturation
Control	-	0.18±0.20	-
Tabernaemontana divaricata flower extract	500	0.0714±1.31	56.7±1.2
	100	0.0497±1.12	43.4±2.1
Ibuprofen	100	0.82±0.6	81.6±1.2
	10	1.14±0.8	61.7±1.3

Table no: 02 Percentage inhibition of HRBC denaturation

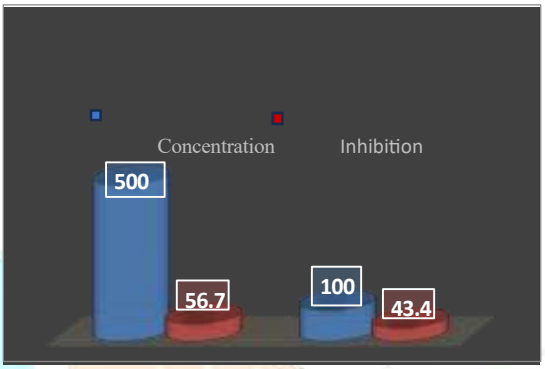

Fig : Percentage inhibition of denaturation in *Tabernaemontana Divaricata* -HRBC METHOD

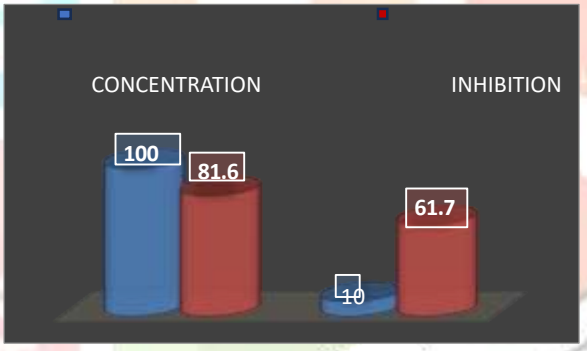

Fig : Percentage inhibition of denaturation in Ibuprofen -HRBC METHOD

PROTEIN DENATURATION:

TYPE OF EXTRACT	CONCENTRATION µg/ml	ABSORBANCE	%INHIBITION OF DENATURATION
Control	-	1.12±1.20	-
Tabernaemontana divericata flower extract	1000	0.715±1.0	73.2±2.1
	500	0.71±1.0	61.5±2.0
	100	0.70±0.5	46.2±2.0
	10	0.05±0.5	31.3±1.8
Ibuprofen	500	0.38±0.52	90.5±1.32
	100	0.38±0.5	82.4±1.31
	10	0.06±0.8	74.1±1.29

Table no :03 Percentage Inhibition of Denaturation

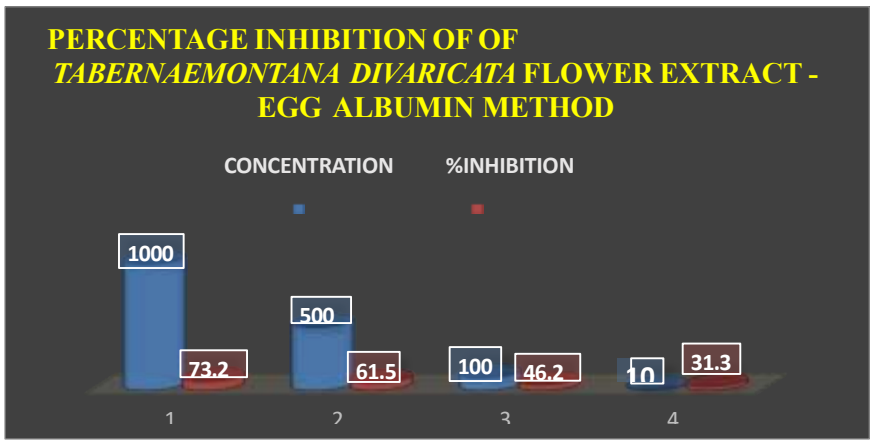

Fig: Percentage Inhibition of PROTEIN DENATURATION - Sample

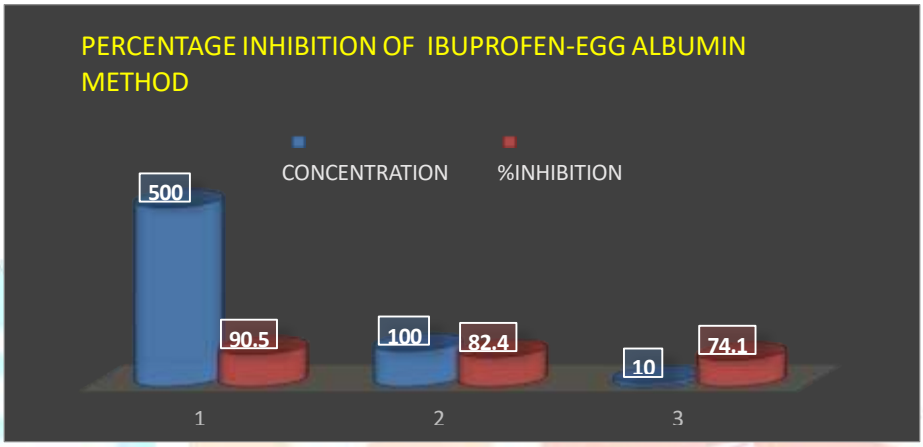

Fig:Percentage Inhibition of PROTEIN DENATURATION – Reference

DISCUSSION

Tabernaemontana divaricata is a highly useful ethno-medicinal plant utilized for various traditional treatment purposes worldwide and contains chemical constituents such as alkaloids, flavonoids, tannins, glycosides and terpenoids. These compounds contribute to the plant's medicinal properties and are responsible for its diverse traditional uses in treating treat fever, respiratory ailments, digestive issues, anti-oxidant, anti- microbial and anti – inflammatory properties. In order to provide a scientific rationale for the plants we looked into the anti-inflammatory therapeutic properties of the flower extract, performed chemical analyses, and compared them to control group and standard drugs.

The extract exhibited the presence of alkaloids, flavonoids, tannins, glycosides as per the phytochemical study performed indicating a key role in the reduction of inflammation.

Proteins act as a fuel supply for the human body because they are an essential food. When proteins are exposed to heat, an organic solvent, a strong acid or base or a concentrated inorganic salt, their tertiary and secondary structure, as well as their biological functions, are lost.

Denaturation of protein has an unpredictable mechanism which includes modification in electrostatic hydrogen, hydrophobic and disulfide bonding. Denaturation of protein causes the production of auto antigens in conditions such as rheumatic arthritis, cancer and diabetes which are conditions of inflammation. **Hence, by inhibition of protein Denaturation, inflammatory activity can be inhibited**.

Plant extracts with anti- inflammatory activity were tested for their ability to prevent protein denaturation, which was used to investigate the mechanism of action for anti-inflammatory activity. In the present study, Ibuprofen was used as reference drug. NSAIDs prevent inflammation by blocking the cyclooxygenase enzyme activity. Anti- inflammatory activity of *Tabernaemontana divaricata* has been reported in several studies.

The flower extract of *Tabernaemontana divaricata* at different concentrations (100, 500 mg/mL) showed significant stabilization towards HRBC membranes.

The percentage protection of methanolic extract at concentration 500 µg/mL was higher than that of other concentrations. However; the percentage protection was found to be lesser then the reference concentration

of Ibuprofen (100, 10 µg/mL) which is a clear indication of inhibition of protein denaturation process by the test extract.

The protein denaturation through Egg Albumin was effectively inhibited when the flower extract of *Tabernaemontana divaricata* was given at different concentrations of 1000, 500, 100 and 10 µg/ml, compared with the standard drug Ibuprofen at 500, 100 and10 µg/ml concentrations.

The results supported the traditional use of this plant in some painful and inflammatory conditions. Because of the presence of biologically active principles, i.e. alkaloids, flavonoids, tannins, glycosides and terpenoids in the same plant from phytochemical investigation.

CONCLUSION

In the current investigation, the *invitro* anti-inflammatory activity of ethanolic flower extract of *Tabernaemontana divaricata* was observed. While plant established herbal medicine has been utilized for generations, the efficiency of such medicines should be proved scientifically and experimentally. Elementary phytochemical examination revealed the existence of many phytochemical components in *Tabernaemontana divaricata.*

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. Secondary metabolites in the plant species, such as alkaloids, flavonoids, phenols, and proteins, may contribute to the biological effect of reducing inflammation.

Given the presence of many metabolites, or active chemical constituents, the need for proper isolation using standard techniques and equipment in different fields to find new lead compounds in drug research should be emphasized. When compared to control, placebo, and standard drug, the ethanolic flower extract of the species showed the most therapeutic activity against inflammation.

We concluded that the ethanolic flower extract of *Tabernaemontana divaricata* have therapeutic efficacy for anti-inflammatory activities invitro after analysing the findings. As a result, herbal medicine has opened up new possibilities for developing broad-spectrum uses of *Tabernaemontana divaricata* well as laying the groundwork for developing new novel potent drugs to treat inflammation.The result indicated that the fruit extract of *Tabernaemontana divaricata* at various concentrations has significant anti-inflammatory property.

The present result indicates the efficacy of *Tabernaemontana divaricata* as an effective therapeutic agent in the treatment of acute inflammation. Further and detailed study are in process for the isolation of active constituent responsible for this property and to identify the possible mechanisms of anti-inflammatory properties

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