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# Preliminary phytochemical screening and in vitro evaluation of anti-inflammatory, anti-arthritic and anti-diabetic activities of various extracts of Bridelia retusa fruit

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***ABSTRACT***

***Aim:*** *the aim of present study of investigation was carry out by preliminary phytochemical screening followed by evaluation of the in vitro anti-inflammatory, anti-arthritic and anti-diabetic activities of Bridelia retusa fruit.*

***Methods:*** *phytochemical screening was done to observe the presence of different secondary metabolites of the plant. The anti-inflammatory activity was evaluated by HRBC method, membrane stabilizing activity was examined by using hypotonic solution prompted human erythrocyte lysis model. Egg albumin and bovine serum albumin (BSA) was used to evaluate the anti-arthritic potential. Alpha-amylase & alpha-glucosidase enzyme was used to evaluate the anti-diabetic activity.*

***Results and Discussion:*** *phytochemical tests of methanol extract of Bridelia retusa fruit showed the presence of flavonoids, alkaloids, steroids, glycosides, saponins, phenols, tannins, volatile compounds, carbohydrates, and cardiac glycosides when compared to other extracts. In case of anti-inflammatory activity the maximum percentage inhibition of membrane stabilization was observed as 87.0 % at 500 µg/ml concentration. The maximum percentage inhibition by protein denaturation and egg albumin method where observed as 90.1% and 92.2% respectively at 500 µg/ml concentration for anti-arthritic activity. The maximum percentage inhibition by α-amylase and α-glucosidase enzyme method was observed as 76% and 70% respectively at 500µg/ml concentration for anti-diabetic activity.*

***Conclusion:*** *Present results highlight the role of various extracts of Bridelia retusa fruit for its anti-inflammatory, anti-arthritic and anti-diabetic activities. It reveals that the phytochemical constituents are responsible for these activities.*

***Key words:*** *Bridelia retusa fruit, anti-inflammatory, anti-arthritic, anti-diabetic, HRBC method, protein denaturation, egg albumin, bovine serum albumin, α-amylase enzyme & α-glucosidase enzyme.*

## INTRODUCTION

Rheumatoid joint inflammation (RA) is an immune system disorder determined by the destruction of ligament and bone, irritation, resultant of destruction, for example deformation of joints or synovial multiplication. RA is the most widely recognized inflammatory joint illness in people and has for some time been grouped among the immune system diseases in which skeletal complexities start with central disintegration of ligament followed by marginal and subchondral bone misfortune. Expanded joint destruction with ankylosis and summed up bone misfortune is feature for late complexities [1]. These drawn out skeletal complexities have unaffected results as they can lead not exclusively to painful joint deformations but also to advanced functional inability and expanded death rates [2]. The in vivo denaturation of proteins may cause the formation of auto antigens in certain arthritic infections [3]. The modification in electrostatic, hydrogen, hydrophobic, and disulphide bonding is included for the system of denaturation [4]. The anti-arthritic activity happens by inhibiting denaturation of protein and membrane lysis and controlling the formation of auto antigen in rheumatic disease. Hence, in vitro anti-arthritic joint action, the inhibition of protein denaturation and membrane lysis was considered. Diabetes mellitus is a clinical syndrome characterized by inappropriate hyperglycaemia caused by a relative or absolute deficiency of insulin or by a resistance to the action of insulin at the cellular level. It is the most common endocrine disorder, affecting 16 million individuals in the United States and as many as 200 million worldwide [5]. Diabetes mellitus is considered to be a serious endocrine syndrome. search for newer drugs continues because the existing synthetic drugs have several limitations. In recent times there has been renewed interest in the plant remedies [6].

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Bridelia retusa spreng. syn: bridelia breezy shawii (family: euphorbiaceae) is a little to direct estimated deciduous tree, spinous when youthful with the grey bark, found all through India up to height of 1000 m aside from in fluctuate dry locations. Regularly, it is significant as astringent, utilized in joint inflammation issues, urinary contamination, anti-fertility and wound healing. The plant parts are utilized to treat dysentery, diarrhoea and diabetes. Leaves and fruits are utilized as antifungal and for stomach ache. It is distinguished additionally for the presence of tannins [7].

In general phytosterols, triterpenoid and tannins have been reported to display anti-inflammatory, anti-ulcer and anti-arthritic properties. The objective of the present work was to investigate the anti-arthritic, anti diabetic and anti-inflammatory effects of Bridelia retusa fruit.

## MATERIALS AND METHODS

**Collection of plant material:**

Bridelia retusa fruits were collected from koringa area of east Godavari Dt., of andhrapradesh the samples were properly confirmed by senior botanist and taxonomist.

**Plant Extract preparation:**

The collected fruits were dried in the shadow until totally dried. At that point the dried fruits were powdered in the blend processor. Sequential extraction was finished utilizing hexane, chloroform,water and methanol. The filtrates were concentrated by removing the solvents under reduced pressure, at 40C, utilizing a rotary evaporator. At the same time, the aqueous extract of the fruits was set up by adding boiled water to the powdered fruits in a measuring beaker on water bath with occasional stirring for 4 hrs. The aqueous extract was separated and reduced under pressure.

**Phytochemical analysis:**

The extracts of Bridelia retusa fruits were analysed for alkaloids, tannins, glycosides, steroids, flavonoids, saponins utilizing standard system procedures.

**Test for Glycosides:** To 1ml of the concentrate was added 2ml of acidic acid and afterward cooled in an ice shower at 4OC. To this mixture 1ml of concentrated tetraoxosulphate acid (H2SO4) was added drop wise. The formation of an oil layer on upper part of solution showed the presence of glycosides [8].

**Test for Alkaloids:** To 3ml of the sample extract was added 1ml of 1% HCL. This subsequent sample mixture was then treated with few drops of Meyer's reagent. The presence of a creamy white precipitate indicated the presence of alkaloids [9].

**Test for Saponins:** Five drops of olive oil was added to 2ml of the plant sample extract and the mixture shaken vigorously. The development of a steady emulsion indicated the presence of saponins [10]

**Test for Tannins:** Two drops of 5% FeCl3 was added to 1ml of the plant sample extract. The presence of a dirty green precipitous indicated the presence of tannins [10].

**Test for Flavonoids:** To 1ml of the plant extract was added 3 drops of ammonia solution followed by 0.5ml of concentrated HCl. The resultant pale earthy colouration of the whole mixture showed the occurrence of flavonoids [8].

**Test for Steroids:** To 1ml of the plant extract was added 1ml of concentrated H2So4. A red colouration showed the occurrence of steroids [10].

**Chemicals and instruments:**

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**Drugs used in the present study include**: Sodium hydroxide, Potassium chloride, Dextrose, Dimethyl formamide, Acetyl salicylic acid, Bovine serum albumin, methanol, chcl3, hexane, Potassium dihydrogen phosphate, Sodium chloride, Disodium hydrogen phosphate, Hydrochloric acid, Sodium citrate, Glibenclamide and distilled water.

**Instruments used were:** Digital photoactometer, $P^H$ meter, UV Spectrophotometer.

## EVALUATION OF ANTIARTHRITIC ACTIVITY

**Bovine serum Albumin Method:**

The reaction sample mixture (0.5ml) contained 0.45 ml bovine serum albumin (5% fluid solution and 0.05 ml of Bridelia retusa extracts at various concentrations (100-500µg/ml). The samples were incubated at 37C for 30 min. After cooling the samples, 2.5 ml phosphate buffer saline (pH 6.3) was added to each tube. Turbidity was estimated spectrophotometrically at 660nm for control test 0.05 ml distilled water was utilized rather than extracts while product control test needed bovine serum albumin. The percentage inhibition of protein denaturation was determined as follows and the results shown in the **table: 1**

% Inhibition of protein denaturation =100 – [{(O.D of test arrangement – O.D of product control)/O.D of test control} × 100]

Control states to 100% protein denaturation. The results were compared with Diclofenac sodium.

**Table 1:** inhibition of different extracts of bridelia retusa fruit on protein denaturation

| Sr.No | Concentration µg/ml | % inhibition on Protein denaturation | | | | |
|---|---|---|---|---|---|---|
| | | Hexane Extract | Chcl3 Extract | Methanol Extract | Aqueous extract | Standard diclofenac sodium |
| 1 | 100 | 24.8 | 30.2 | 35.5 | 20.4 | 38.3 |
| 2 | 200 | 32.2 | 45.2 | 53.2 | 31.4 | 55.5 |
| 3 | 300 | 51.0 | 60.9 | 64.7 | 48.3 | 74.0 |
| 4 | 400 | 68.4 | 72.3 | 81.7 | 57.8 | 82.3 |
| 5 | 500 | 79.3 | 81.0 | 90.1 | 69.4 | 93.9 |

**Egg Albumin Denaturation:**

The test mixture comprised of different extracts of Bridelia retusa fruit at various concentrations i.e.; 100 µg/ml to 500 µg/ml and 1% watery bovine albumin solution. Precisely pH of the reaction sample mixture was changed in accordance with 6.4 utilizing 1NHCl. The samples were permitted to incubate at 37˚C for 20min and afterward heated at 57˚C for 20min. Subsequent to cooling; their absorbance was estimated at 660 nm by utilizing unadulterated blank. Diclofenac sodium (standard medication) was utilized as reference drug and rewarded as such for assurance of absorbance [11]. The percentage inhibition of protein denaturation was determined as follows and the results shown in the **table: 2**

Percent inhibition = (Abs control-Abs treated)/ (Abs treated) ×100

**Table 2: inhibition of different extracts of Bridelia retua fruit on egg albumin denaturation**

| Sr.No. | Concentration µg/ml | % inhibition on egg albumin denaturation | | | | |
|---|---|---|---|---|---|---|
| | | Hexane extract | Chcl3 extract | Methanol Extract | Aqueous extract | Standard diclofenac sodium |
| 1 | 100 | 28.6 | 33.3 | 39.8 | 27.6 | 40.5 |
| 2 | 200 | 37.6 | 42.7 | 51.3 | 35.6 | 58.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 3 | 300 | 60.4 | 63.4 | 72.6 | 49.4 | 76.3 |
| 4 | 400 | 74.3 | 77.3 | 83.3 | 65.3 | 89.1 |
| 5 | 500 | 85.5 | 88.4 | 92.2 | 72.5 | 95.5 |

## EVALUATION OF ANTI INFLAMMATORY ACTIVITY

**HRBC Membrane Stabilization Method:**

The erythrocyte layer takes after to lysosomal membrane and accordingly, the impact of medications on the adjustment of erythrocyte could be extrapolated to the adjustment of lysosomal membrane. In this manner as the layer stabilizes, it interferes with the discharge or potentially activity of mediators like histamine, serotonin, prostaglandins, and leukotriene's which are responsible for irritation. The anticipation of hypo tonicity induced HRBC membrane lysis is taken as a measure of Anti-inflammatory action.

**Preparation of Human Red Blood Cells (HRBC) Suspension:**

New entire human blood was grouped and blended in with equivalent volume of disinfected Alsever solution (2 % dextrose, 0.8 % sodium citrate, 0.05% citrus extract and 0.42 % sodium chloride in water). The blood was centrifuged at 2600 rpm for 10 min and stuffed cells were washed multiple times with isosaline (0.85%, pH 7.2). The volume of the blood was estimated and reconstituted as 10% v/v suspension with isosaline.

**Heat induced haemolysis:**

The reaction sample mixture (4.5 ml) comprises of 2ml of hypo saline (0.25% w/v NaCl), 1 ml of 0.15 M phosphate buffer (pH 7.4) and 1 ml of test solution (100µg/ml to 500µg/ml) in isosaline, 0.5 ml of 10% HRBC in isosaline was added. For test control, 1 ml of distilled water utilized rather than hyposaline (to deliver 100% haemolysis), while product control needed red platelets. The sample mixtures were incubated at 37˚C for 30 min and centrifuged at 3000 rpm 20 min. Diclofenac sodium was utilized as the reference drug. The haemoglobin content in the suspension was evaluated utilizing a spectrophotometer at 560 nm [12]. Percentage membrane stabilizing action was determined as follows and the results are shown in **table: 3**

**Table 3:** percentage stabilization of different extracts of Bridelia retusa fruit using HRBC method

| Sr.No | Concentration µg/ml | % stabilization on HRBC method | | | | |
|---|---|---|---|---|---|---|
| | | Hexane extract | Chcl3 Extract | Methanol Extract | Aqueous extract | Standard diclofenac sodium |
| 1 | 100 | 21.8 | 25.5 | 28.2 | 19.4 | 34.3 |
| 2 | 200 | 30.2 | 37.2 | 39.2 | 28.4 | 52.5 |
| 3 | 300 | 45.0 | 49.7 | 50.9 | 40.3 | 68.0 |
| 4 | 400 | 59.4 | 61.7 | 72.3 | 58.8 | 80.3 |
| 5 | 500 | 70.3 | 75.1 | 87.0 | 65.4 | 91.9 |

Formula:

% Membrane stabilization = (Abs control-Abs rewarded)/ (Abs treated) ×100

## EVALUATION OF ANTI-DIABETIC ACTIVITY

**Inhibition of alpha-amylase enzyme:**

Starch preparation (0.1% w/v) was set up by mixing 0.1g of potato starch in 100 ml of 16 mM of sodium acetic acid derivation buffer. The enzymatic compound solution was set up by blending 27.5 mg of alpha-amylase in 100 ml of distilled water. The colorimetric reagent was set up by blending sodium potassium tartarate solution and 3, 5 Di-nitro salicylic acid solution at 96 mM fixation. Both control and plant extracts were independently

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included with starch solution and left to respond with alpha-amylase solution under basic conditions at 25°C. Their activity was estimated following 3 minutes. The group of maltose was measured by the decrease of 3, 5 Di-nitro salicylic corrosive to 3-amino-5-nitro salicylic. This response is visible at 540 nm. The results are shown in the **table 4:**

**Table 4:** percent inhibition of different extracts of Bridelia retusa fruit using α-amylase enzyme

| Sr.No | Concentration µg/ml | Percent inhibition | | | | |
|---|---|---|---|---|---|---|
| | | Hexane Extract | Chcl3 Extract | Methanol Extract | Aqueous Extract | Standard glibenclamide |
| 1 | 100 | 19.3 | 24.5 | 30.4 | 18.4 | 42.1 |
| 2 | 200 | 25.4 | 38.0 | 41.9 | 22.3 | 55.5 |
| 3 | 300 | 32.6 | 43.1 | 52.2 | 30.8 | 76.5 |
| 4 | 400 | 45.3 | 56.3 | 62.8 | 42.6 | 80.0 |
| 5 | 500 | 58.3 | 69.5 | 76.0 | 63.0 | 85.2 |

**Inhibition of Alpha-glycosidase Enzyme:**

The inhibitory measure of alpha-glucosidase protein was dictated by producing 1 ml sample preparation of starch substrate (2 % w/v maltose or sucrose) with 0.2 M Tris support pH 8.0 and plant removes independently for 5 minutes at 37°C. Their activity was started by including 1 ml of alpha-glucosidase protein (1U/ml) to it followed by producing for 40 minutes at 35°C. At that point the response was ended by the expansion of 2 ml of 6N HCl. At that point the intensity of the colour was estimated at 540nm [13]. The results are shown in the **table 5:**

**Table 5:** percent inhibition of different extracts of Bridelia retusa fruit using α-glucosidase enzyme

| Sr.No | Concentration µg/ml | Percent inhibition | | | | |
|---|---|---|---|---|---|---|
| | | Hexane Extract | Chcl3 Extract | Methanol Extract | Aqueous Extract | Standard Glibenclamide |
| 1 | 100 | 18.0 | 28.6 | 30.9 | 20.3 | 37.5 |
| 2 | 200 | 26.3 | 36.7 | 42.5 | 31.0 | 49.3 |
| 3 | 300 | 33.2 | 44.3 | 54.1 | 40.6 | 61.8 |
| 4 | 400 | 48.0 | 56.2 | 61.3 | 54.7 | 78.4 |
| 5 | 500 | 59.1 | 67.2 | 70.0 | 62.5 | 81.0 |

## RESULTS

**Phytochemical screening:**

Different types of chemical tests are carried out and the Preliminary phytochemical tests of methanol extract showed the positive result of flavonoids, alkaloids, steroids, terpenoids, amino acids, carbohydrates, volatile compounds, tannins, saponins and glycosides and the result shown in the **table 1:**

**Anti-arthritic activity (table and figure)**

Bridelia retusa fruit extract showed significant activity (79.3, 90.1, 81.0, 69.4)on bovine serum albumin method and showed maximum effect (85.5, 88.4, 92.2, 72.5) on egg albumin denaturation method in different extracts based on total anti-arthritic activity is as follows: methanol >chcl3> hexane> aqueous extract when compared to standard diclofenac sodium (93.9 and 95.5). Table 2, 3 and figure 1, 2 shown as inhibition of different extracts of Bridelia retusa fruit on protein denaturation.

**Anti-inflammatory activity:**

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Bridelia retusa fruit extract showed significant activity (70.3, 75.1, 87.0, 65.4) on HRBC method in different extracts based on total anti-inflammatory activity is as follows: methanol> chcl3 > hexane> aqueous extract when compared to standard diclofenac sodium (91.9). Table 4 and figure 3 shown as the following result of percentage stabilization of different extracts of Bridelia retusa fruit on HRBC method.

**Anti diabetic activity:**

Bridelia retusa fruit extract showed significant activity (58.3, 69.5, 76.0, 63.0 and 59.1, 67.2, 70.0, 62.5) on α-amylose and α-glucosidase enzyme in different extracts based on total anti diabetic activity is as follows: methanol>chcl3>aqueous extract>hexane when compared to standard GLB(glibenclamide 85.2 & 81.0) the result shown as table 5,6 and figure 4,5.

**Table 1. Phytochemical constituents of Bridelia retusa fruits**

| phytochemicals | Hexane extract | Chcl3 extract | Methanol extract | Aqueous extract |
|---|---|---|---|---|
| Alkaloids | + | + | + | + |
| Amino acids | + | + | + | + |
| Anthraquinone | - | - | + | - |
| Carbohydrate | + | + | + | + |
| Cardiac glycosides | - | + | + | - |
| Flavonoids | + | + | + | + |
| Glycosides | + | + | + | + |
| Phenols | - | + | + | - |
| Saponins | - | + | + | - |
| Steroids | + | + | + | + |
| Tannins | - | - | + | + |
| Terpenoids | + | + | + | - |
| Volatile compounds | + | + | + | + |

(+); Presence – (-); Absence

The presence of these metabolites recommends maximum potential for the plant as a source of helpful phytomedicines [14].

## DISCUSSION

Inflammation is a response of living tissue towards injury. Plants have been utilized because of different phyto-chemicals synthesized as secondary metabolites. Plant extracts and phyto-chemicals can be of great significance in medical treatments. The compounds are known by their active substances like phenols, alkaloids, and tannins etc. [15]. Methanol extract showed maximum effect due to the presence of all phytochemicals like flavonoids, alkaloids, steroids, terpenoids, carbohydrates, amino acids, anthraquinone, cardiac glycosides, phenols, tannins, saponins, volatile compounds and glycosides. In the present research study, in vitro anti-inflammatory, anti-arthritic and anti-diabetic activities was studied. The anti-inflammatory and anti-arthritic activity was performed by using HRBC membrane stabilization method, egg albumin denaturation method and protein denaturation method. Similarly the anti-diabetic activity was performed by using inhibition of α-amylase enzyme and inhibition of α-glucosidase enzyme. As part of the analysis on the mechanism of the anti-arthritic activity, ability of plant extract to inhibit protein denaturation was studied. It was effective in inhibiting heat induced albumin denaturation. Methanol extracts of *Bridelia retusa fruit* are capable of controlling the production of auto antigens and inhibits denaturation of protein and its effect was compared with the standard drug diclofenac sodium the percent inhibition was found to be diclofenac sodium. The Bridelia retusa fruit extract showed dose dependent manner. This effect may be due to the presence of alkaloids, flavonoids and steroids present in various fractions.

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The protein and egg albumin denaturation activity of the methanol extract of Bridelia retusa fruit studied to be effective at 90.1% & 92.2% (500µg/ml) when compared to that of standard drug diclofenac sodium 93.9 % &95.5% (500µg/ml) respectively as shown in **fig1&2.**

The results of HRBC assay of the methanol extract of Bridelia retusa fruit studied to be effective at 87 .5% (500 µg/ml) when compared to that of standard drug diclofenac sodium 91.1% (500µg/ml) as shown in **fig3.**

The in vitro anti-diabetic method involves the inhibition of both α-amylase and α-glucosidase enzyme in a dose dependent manner. The α-amylase and α-glucosidase enzyme activity of the methanol extract of Bridelia retusa fruit studied to be effective at76 % and70 % (500 µg/ml) when compared to that of standard drug Glibenclamide 85.2%and81% (500 µg/ml) **fig4&5.**

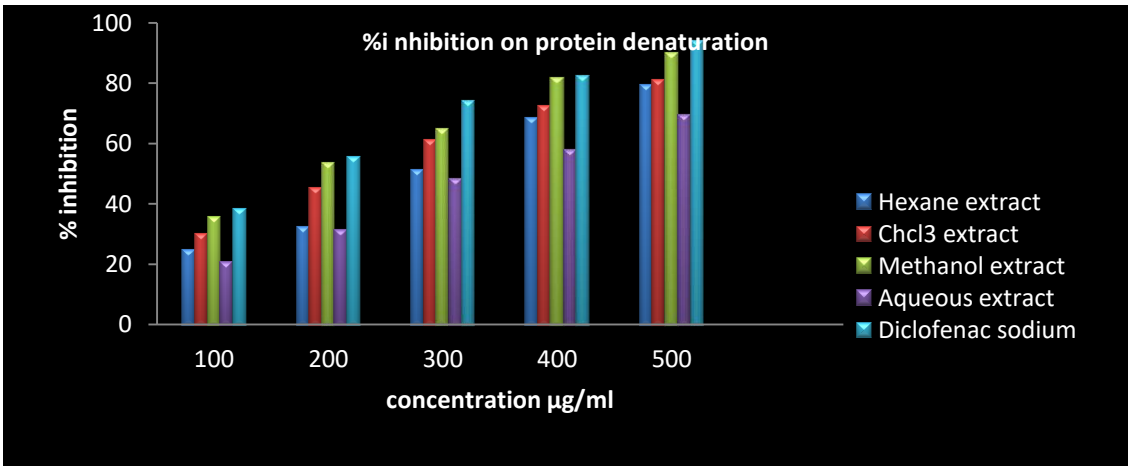


**Figure 1:** percent inhibition on protein denaturation using different extracts of Bridelia retusa fruit

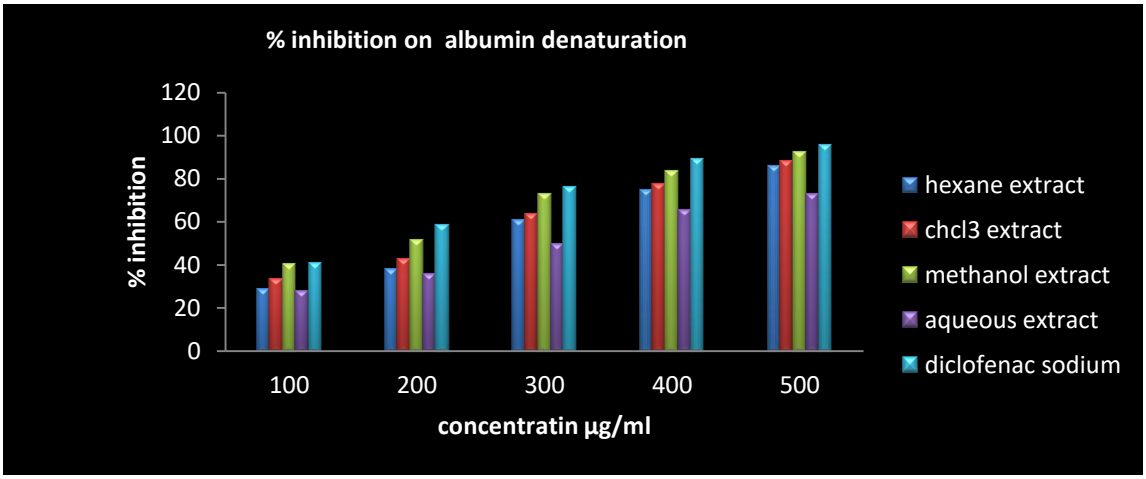


**Figure 2:** percent inhibition on albumin denaturation using different extracts of Bridelia retusa fruit

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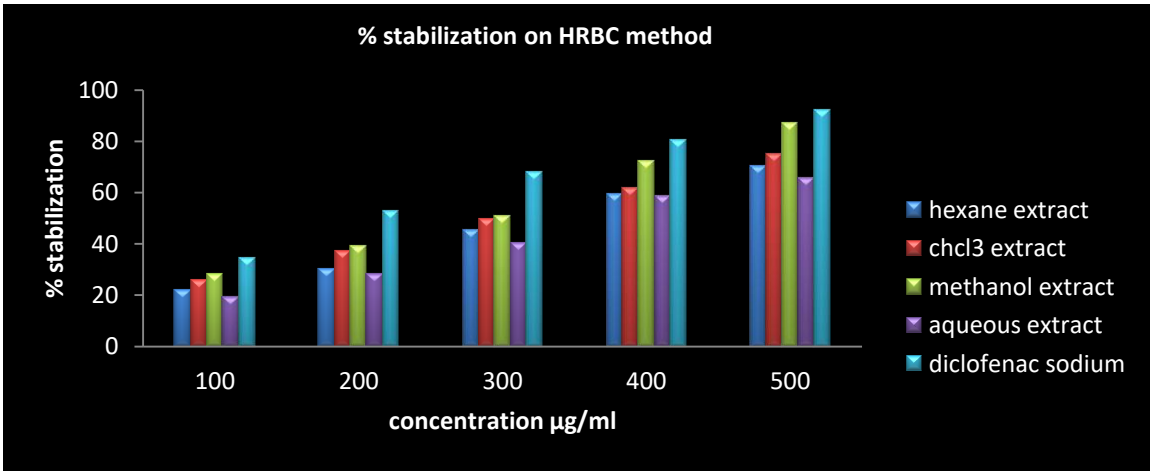


**Figure 3: percent** stabilization on HRBC method using different extracts of Bridelia retusa fruit

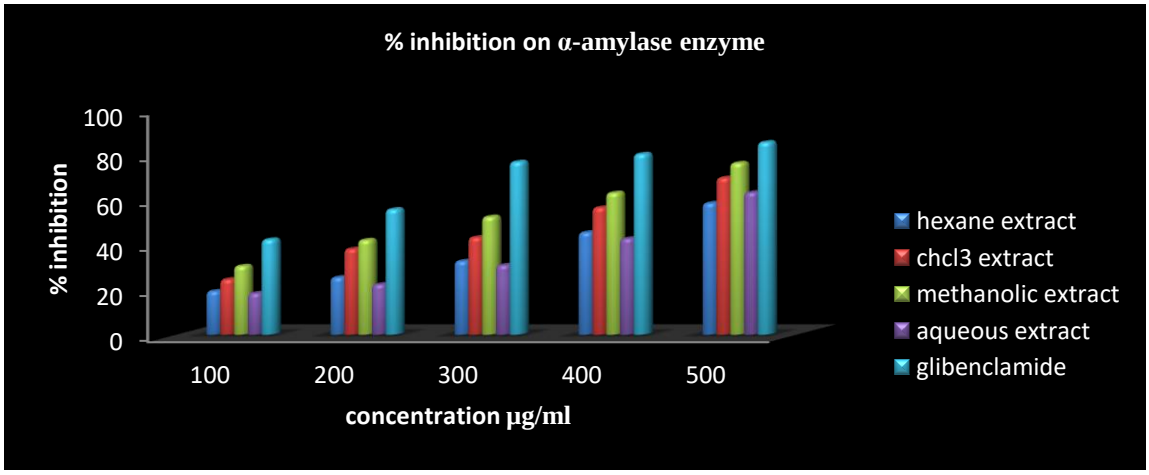


**Figure 4:** percent inhibition on α-amylase enzyme using different extracts of Bridelia retusa fruit

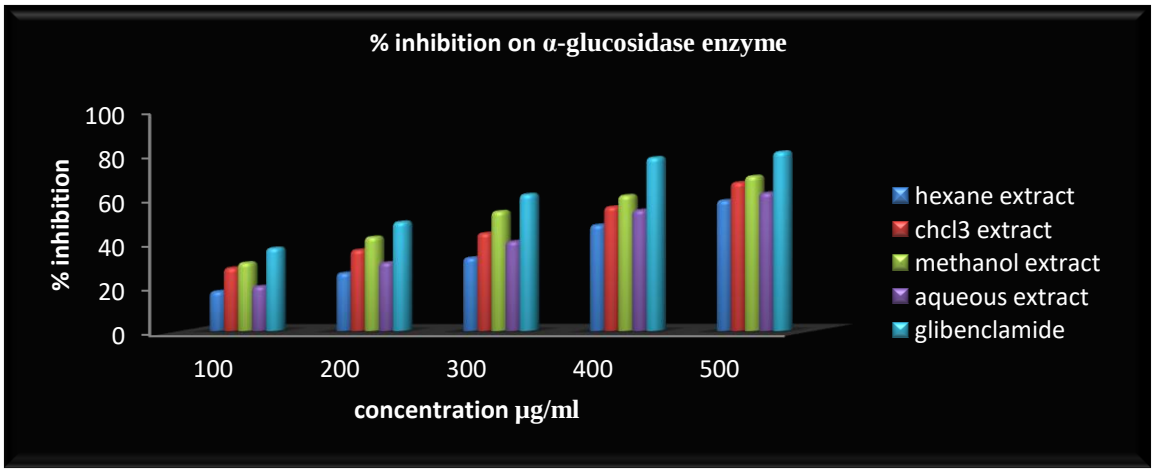


**Figure 5:** percent inhibition on α-glucosidase enzyme using different extracts of Bridelia retusa fruit

## CONCLUSION

From the results of the current study it can be stated that bridelia retusa fruit is capable of controlling the production of auto antigens due to in vitro denaturation of proteins in rheumatic diseases. Hence, from the

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obtained results, it can be concluded that bridelia retusa fruit extracts possesses anti-inflammatory, anti-diabetic and anti-arthritic activities. The obtained results, it can be concluded that bridelia retusa fruit extract possesses anti-arthritic activity. However, the methanol and chloroform (chcl3) extracts exhibited significant activity when compared with the standard drugs diclofenac and glibenclamide. . Further mechanistic studies are required to isolate, purify and analyse the specific bioactive compound respectively for the anti-arthritic activity.

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