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RESEARCH ARTICLE

Biological Evaluation of *Chukrasia tabularis A. Juss* Leaves for Anti-Diabetic and Anti-Arthritic Activities

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ABSTRACT

The plant under evaluation *Chukrasia tabularis*, a plant of Meliaceae, which is usually found, scattered in evergreen rainforest. From the review of literature, it was reported that various pharmacological activities were reported on *Chukrasia tabularis* A. Juss. So far Anti-Diabetic & Anti-Arthritic activities are not reported on the plant. In view of this, the present study was aimed to carryout biological evaluation of the obtained Ethyl Acetate fraction for Anti-Diabetic (*In vitro* α-Amylase Inhibition Assay with Acarbose as standard and *Ex vivo* Rat Everted Sac Model method with Acarbose as standard) and Anti-Arthritic activities (*In vitro* Protein Denaturation Assay method with Diclofenac sodium as standard) respectively. The results were compared with the standard using statistical method. The Anti-Diabetic activities of Ethyl acetate fraction showed significant activity when compared to standard. The Anti Arthritic activity of the Ethyl acetate showed more protection activity than the standard.

KEYWORDS

Chukrasia Tabularis, Flavonoids, Meliaceae, Anti-Diabetic, Anti-Arthritic

INTRODUCTION

Diabetes is diseases in which a person has high blood sugar do not respond to the insulin that is produced. Diabetes mellitus affects approximately 5 to 8% of the population. . Diabetes was one of the major killers of mankind with an estimate of minimum 40 million people suffering from this disorder. In this disease blood glucose level rises above 80-120 mg/dl. A large number of individuals are asymptomatic and do not know they have the disease. Hyperglycemia is a common end point for all types of diabetes mellitus and is the parameter that is measured to evaluate and manage the efficacy of diabetes therapy.

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One therapeutic approach which may prove to be beneficial for treatment of diabetes is to decrease the post-prandial hyperglycemia. This can be achieved by retarding the absorption of glucose through the inhibition of the carbohydrate hydrolyzing enzymes in the digestive tract. Therefore, it is the need of time to identify and explore the amylase inhibitors from natural sources having fewer side effects. In recent year the emphasis has been to identify as many plants as possible which could have effective control of the disease. Pharmacological screening and clinical trial reveal the presence of hypoglycemic activity in large number of plants.

Rheumatoid arthritis primarily affects the lining of joints, but can also affect other parts of the body in more than 15-25% of the individuals. Arthritis is a form of joint disorder that involves inflammation of one or more joints. The most common form, osteoarthritis (degenerative joint

disease), is a result of trauma to the joint, infection of the joint, or age. The major complaint by individuals who have arthritis is joint pain. The pain from arthritis is due to inflammation that occurs around the joint, damage to the joint from disease, daily wear and tear of joint, muscle strains caused by forceful movements against stiff painful joints and fatigue. By controlling the production of auto antigen and inhibiting denaturation of protein in rheumatic disease leads to anti-arthritic activity. Rheumatoid arthritis has no complete cure but the symptoms can be treated and the progress of the disease can be slowed down with the help of drugs. Mild and early cases are still mostly treated with NSAIDs while disease modifying antirheumatic drugs is currently being recommended. Hence, we are in a need to find an alternative medicine in the treatment of RA with less side effects and toxicity.

To solve these problems, new approaches has been urgently needed on the use of plant based natural products. *Chukrasia*[1] is usually found scattered in evergreen rainforest, moist semi-evergreen forest and mixed deciduous forest at altitudes from 20 m to 1450 m. From the literature review plant was reported to be rich in Limonoids, Phragmalin derivatives, Tannins, Flavonoids and other Phenolic Compounds having various pharmacological activities. In view of this, the present study was aimed to carryout biological evaluation of the obtained Ethyl Acetate fraction for Anti-Diabetic and Anti-Arthritic activities respectively.

MATERIALS AND METHODS

Collection and Authentication

Young leaves of *Chukrasia tabularis* A. Juss were collected from Dharavarithota, Ongole. The plant was authenticated by Department of Botany, Hindu College, Guntur.

Preparation of Plant Extract[2,3,4]

The leaves of the plants were removed from the plants and then washed under running tap water to remove dust. The plant samples were then air dried for few days and the leaves were crushed into powder and stored in polythene bags for use. The coarse powdered leaves of the *Chukrasia tabularis* were initially extracted with Methanol. The obtained extracts were then subjected to preliminary phytochemical evaluation to know the compounds present in the extracts. The methanolic extract was taken into a beaker and was dissolved in distilled water. The obtained aqueous methanolic extract was then transferred into a separating flask. Initially, Ethyl acetate was added to the aqueous methanolic extract. The separatory flask was then shaken vigorously and later the liquids were collected separately. The process was repeated thrice with the same solvent and the Ethyl acetate fractions are pooled together. The obtained extract was then subjected to Solvent-Solvent Separations Method[5] by passed through column chromatography and isolation of chemical constituents was done. The Ethyl acetate fraction of the methanolic extract was estimated for total flavonoid content present in it and was found to contain 180 mcg of Flavonoids which are responsible for the Anti-Diabetic and Anti- Arthritic activities respectively.

Evaluation of Anti- Diabetic Activity[6,7]

In Vitro a -Amylase Inhibition Assay Method[8]

Preparation of the Reagents

1. Reagent A: To dissolve 20mM sodium phosphate with 6.7mM sodium chloride in 100ml deionized water. The pH was adjusted to 6.9 at 20°c with 1M sodium hydroxide.

2. Reagent B: To dissolve 1% w/v of potato starch in 25ml of Reagent A. The solubilization is facilitated by heating the starch solution in a glass beaker directly on a heating/stir plate, with constant stirring for 15 minutes.

3. Reagent C: To dissolve 12g of sodium potassium tartrate tetra hydrate in 8ml of 2M sodium hydroxide solution.

4. Reagent D: To dissolve 96mM 3, 5- dinitro salicylic acid in 20ml of deionized water.

5. Reagent E: To Equimolar mixture of Reagent C to Reagent D slowly with stirring. The solution was diluted to 40ml with deionized water.

6. Reagent F: To dissolve 1unit/ml of α- amylase in cold deionized water.

***Procedure*[9,10]**

To 1ml of Reagent B was mixed with 1ml of the Ethyl acetate fraction solution. To this 1ml of Reagent F was added and stand for 3 minutes at 25°C. Then 1ml of colorimetric Reagent E was added. The contents were heated for 10 to 15 minutes on a boiling water bath. The generation of maltose was quantified by the reduction of 3, 5-dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction was measured at 540nm against the reagent blank. The percentage of inhibition was determined by using the formula,

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{test}}{\text{Control}} \times 100$$

***In vitro α* Amylase Inhibitory Assay[11,12]**

The Ethyl acetate fraction of the methanolic leaf extract of *Chukrasia tabularis* A. Juss was tested at 2, 4, 6, 8, 10 mg/ml concentrations in comparison with the standard Acarbose (2, 4, 6, 8, 10 mg/ml). The % inhibition was calculated using the formula as shown in Table 1 and in Figure 1.

Table 1: *In vitro α* Amylase inhibition assay

Concentration (mg/ml)	% Inhibition of α Amylase ± SEM	
	Acarbose (Standard)	Ethyl acetate Fraction
2	22.69±1.26	20.08±1.32
4	34.53±0.81	31.12±0.45
6	43.17±0.90	39.95±1.39
8	54.41±1.67	51.60±0.64
10	79.51±0.87	77.51±1.70
IC 50	0.56	0.97***

N = 5 *** P< 0.005

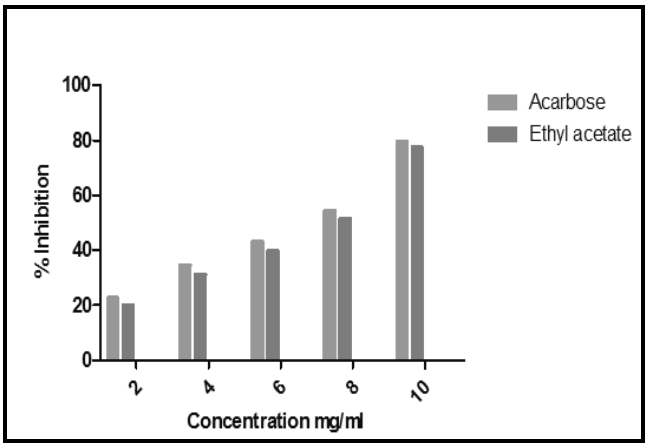

Figure 1: *In vitro α* Amylase Inhibition Assay

In the *in vitro α*-Amylase Inhibitory Assay, the Ethyl acetate fraction was tested along with the standard Acarbose. Though the % inhibition of *α*-Amylase was numerically less than that of the standard, the Ethyl acetate fraction showed significant *α*-Amylase inhibition activity. The inhibition exerted by the Ethyl acetate fraction was also Dose dependent.

***Ex Vivo* Rat Everted SAC Model**

***Experimental Protocol*[13,14]**

Male albino rats of Wistar strain weighing 150-200gm were obtained from Animal House of Hindu College of pharmacy. They were housed in standard cages at room temperature (25 ± 2ºC) and relative humidity (55 ± 5 %) and 12/12 h light/ dark cycle. The animals were provided with standard pellet diet and water ad libitum. All animals are acclimatized for 10 days. The study was performed as per the guidelines of the CPCSEA, Government of India. The proposal number submitted to CPCSEA was HCOP/IAEC/2013-14/03.

***Preparation of Everted Intestinal Sac*[15,16]**

Overnight fasted rats were first anesthetized using light ether and sacrificed. The abdomen was opened by a midline incision. The whole of the small intestine was removed by cutting across the upper end of the duodenum and the lower end of the ileum and manually stripping the mesentery. The small intestine was washed out carefully with cold saline solution (0.9 % w/v NaCl) using a syringe equipped with blunt end. The mid portion of the small intestine from each

animal was used in order to minimize the transport variability of the segments. Intestinal segments (5 ±0.2 cm) were then everted and were filled with 0.5ml of distilled water. The filled intestinal sac was then slipped off the needle carefully and the loose ligature on the proximal end was tightened. The sacs were grouped into 5 groups. A total of 60 sacs were prepared. 12 sacs were placed in group (Control, Test Solution 0.5mg/ml, 1mg/ml, 2mg/ml Standard). The temperature was maintained at 37˚C and proper aeration was provided as shown in Figure 2 and Figure 3.

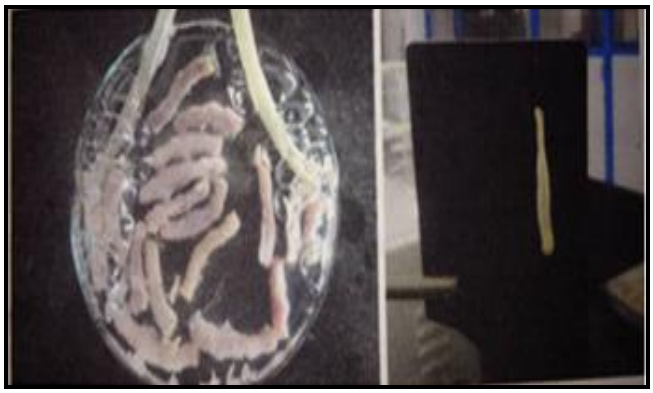

Figure 2: Everted Intestinal SACS

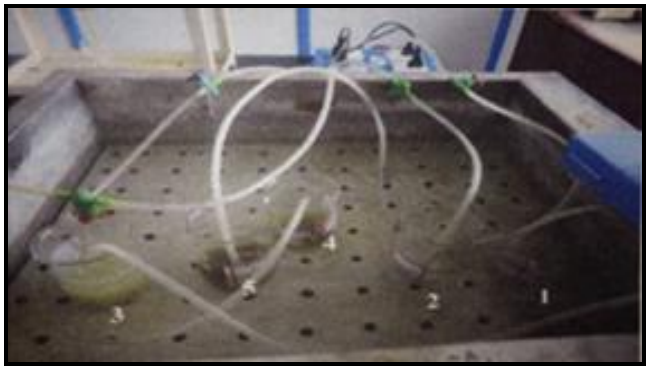

Figure 3: 1) Control, 2) Standard Acarbose, 3) EA (0.5 mg/ml), 4) EA (1mg/ml), 5) EA (2mg/ml)

Intestinal Transport Studies[17,18]

At the end of the incubation period (15, 30, 45, 60 mins) the sac was removed from the organ bath, blotted by a standardized procedure. The contents of the sac were drained through a small incision into a test tube. So as to empty the sac completely, gentle pressure was applied. The glucose content in each sample was analyzed by auto analyzer. At regular intervals of time (15, 30, 45, 60 mins after loading the sacs) 3 sacs from each group were taken out and blotted properly on a blotting paper. The contents from the inside of each sac were collected separately and glucose content was determined using Gluco-kit and auto analyzer. In the *in vitro* α-Amylase Inhibitory Assay, the Ethyl acetate fraction was tested along with the standard Acarbose. Though the % inhibition was numerically less than that of the standard, the Ethyl acetate fraction showed significant α-Amylase inhibition activity. The inhibition exerted by the Ethyl acetate fraction was also Dose dependent.

Ex Vivo Rat Everted Sac Model Assay[19]

The Glucose uptake in the everted sac of the intestine of rats in different solutions (Control, Standard Acarbose (2mg/ml), EA [0.5mg/ml, 1mg/ml, 2mg/ml respectively]) was calculated at 15, 30, 45, 60 mins time intervals. The results obtained were presented below as shown in Table 2 and Figure 4.

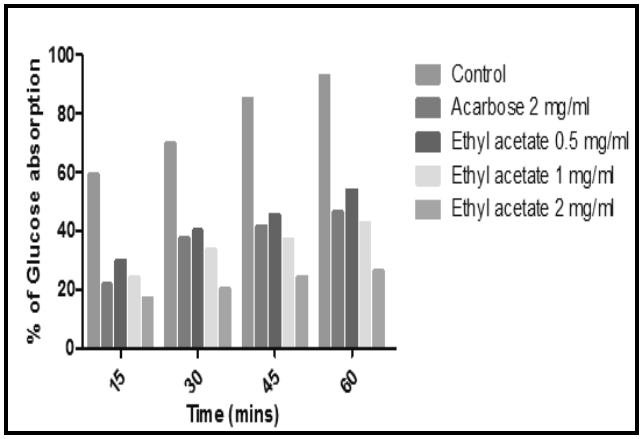

Figure 4: *Ex vivo* depicting % of Glucose absorption

In the *ex vivo* Rat Everted Sac Model, the standard Acarbose (2mg/ml) was compared with Ethyl Acetate (0.5mg/ml), Ethyl Acetate (1mg/ml) and Ethyl Acetate (2mg/ml) test solutions. At equal concentrations of 2mg/ml, ethyl acetate fraction showed potent inhibition of Glucose absorption compared to standard. Whereas, EA 1mg/ml solution significant activity, but was less than that of the standard. EA 0.5 mg/ml showed lesser inhibition of glucose uptake.

Table 2: *Ex vivo* Rat Everted Sac Model

Time	Glucose uptake (mg/ml) ± SEM				
	Control	**Acarbose**	**EA 0.5 mg/ml**	**EA 1 mg/ml**	**EA 2 mg/ml**
15	59.06±1.31	21.70±1.21	29.93±0.6	24.08±0.32	17.13±0.81***
30	69.77±0.44	37.41±0.59	40.12±0.62	33.37±0.46***	20.26±0.42***
45	84.81±1.25	41.50±0.94	45.55±0.87	36.95±1.38***	24.26±0.63***
60	92.88±1.32	46.24±0.95	53.89±0.49	42.63±0.61***	26.32±0.47***

EA = Ethyl acetate fraction
SEM=Standard Error Mean
N= 3 *** P< 0.0001

Evaluation of Anti-Arthritic Activity

***In Vitro Protein Denaturation Assay*[20]**

***Inhibition of Protein Denaturation*[21,22]**

1. Test solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%W/V aqueous solution) and 0.05ml of Ethyl acetate fraction solution.

2. Test control solution (0.5ml) consist of 0.45ml of bovine serum albumin (5%W/V aqueous solution) and 0 .05ml of distilled water.

3. Product control (0.5ml) consists of 0.45ml of distilled water and 0.05 ml of test solution (i.e., Ethyl Acetate fraction)

4. Standard solution (0.5ml) consists of 0.45ml of Bovine serum albumin (5%w/v aqueous solution) and 0.05ml 0f Diclofenac sodium.

Various concentrations (50, 100, 200, 500, 1000, 2000 µg/ml) of plant extracts (test solution) and Diclofenac sodium standard (50, 100, 200, 500, 1000, 2000 µg/ml) were taken respectively. All the above solutions were adjusted to pH 6.3 using 1N HCl. The samples were incubated at 37°c for 20 minutes and the temperature was increased to keep the samples at 57°c for 3 minutes. After cooling, add 2.5 ml of phosphate buffer to the above solutions. The absorbance was measured using UV-Visible spectrophotometer at 416nm.

The control represents 100% protein denaturation. The results were compared with Test and Standard solution and percentage inhibition can be calculated as,

$$\% \text{ Inhibition} = \frac{[100 - (\text{optical density of test solution} - \text{optical Density of product control})] \times 100}{(\text{Optical density of test control})}$$

***In vitro* Protein Denaturation Assay[23]**

Table 3: *In vitro* Protein Denaturation Assay

Concentration	% Protection ± SEM	
	Diclofenac Sodium	**Ethyl acetate Fraction**
50µ/ml	31.11±0.57	78.37±0.67
100µ/ml	41.58±0.36	81.08±0.62
200µ/ml	58.62±1.4	89.18±0.94
500µ/ml	71.19±1.2	91.89±1.32
1000µ/ml	81.35± 0.93	118.91±1.56
2000µ/ml	129.73±0.72	133.96±0.65

SEM = Standard Error Mean N= 3

The Ethyl acetate fraction of the methanolic extract of *Chukrasia tabularis* A. Juss was tested at 50, 100, 200, 500, 1000, 2000 µ/ml concentrations in comparison with the standard Diclofenac Sodium (50, 100, 200, 500, 1000, 2000 µ/ml) for Anti-Arthritic activity as shown in Table 3 and Figure 5.

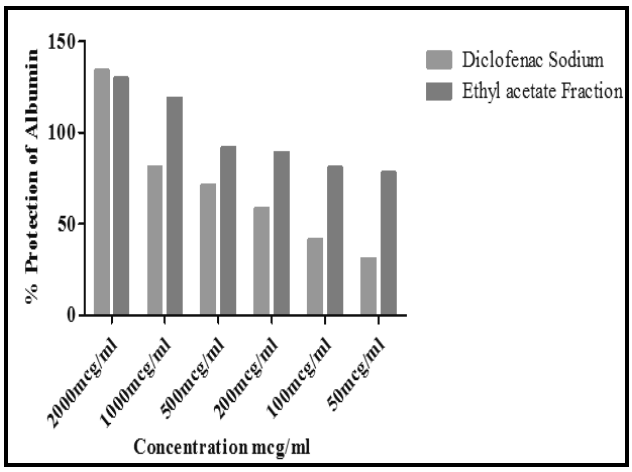

Figure 5: *In vitro* evaluation of Anti- Arthritic Activity by Protein Denaturation Assay

When compared to Acarbose (Standard), Ethyl acetate fraction showed more inhibition of protein denaturation at 50, 100, 200, 500, 1000 mcg/ml concentrations. At 2000 mcg/ml concentration Standard drug Acarbose showed better inhibition of Protein denaturation[24] than the Ethyl acetate fraction.

CONCLUSION

The *Chukrasia tabularis* A. Juss leaves were extracted, separated and isolated. The Ethyl acetate fraction of the methanol extract was found to be rich in Flavonoids. The ethyl acetate fraction was evaluated for possible anti diabetic (*in vitro α* Amylase inhibitory assay & *ex vivo* Rat everted sac model) and Anti-arthritic (*in vitro* Protein denaturation assay) activities. Ethyl acetate fraction showed significant but less *in vitro* α-Amylase inhibition activity when compared to standard. In *ex vivo* Rat everted sac model, Ethyl acetate fraction at 1mg/ml concentration showed significant but less activity compared to standard. Ethyl acetate fraction at 2mg/ml concentration showed potent activity than the standard Acarbose (2mg/ml). Ethyl acetate fraction showed comparable Anti-arthritic activity to that of standard. At initial concentrations of 50, 100, 200, 500, 1000 mcg/ml, Ethyl acetate fraction showed more protection activity than the standard. At 2000 mcg/ml concentration, Standard showed more protection than Ethyl acetate fraction of same concentration.

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