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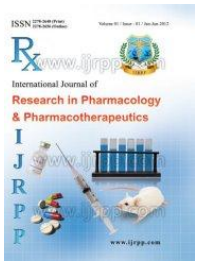

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Study of *invitro* antioxidant, antidiabetic and antibacterial activity of *Clausena anisata* (Willd). Hook (Rutaceae) leaf extracts

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ABSTRACT

Clausena anisata (Willd.) Hook of family Rutaceae, is a deciduous shrub or small tree that contains a wide array of compounds exhibiting diverse range of bioactivity. Leaf extracts are traditionallly used as effective remedies for worms infections, respiratory ailments, hypertension, malaria, fever, rheumatism, arthritis and other inflammatory conditions, headaches, pains, toothaches, convulsions. It has antimicrobial, fungicidal, insecticidal, and antidiabetic activities. The present study has been undertaken to find out the phytochemical, antioxidant, antidiabetic, and antibacterial activity of the leaf extracts of *Clausena anisata* (Willd.) Hook. Leaf was subjected to successive extraction using aqueous, alcohol, acetone, ethylacetate+chloroform and hexane to obtain the respective extracts. DPPH free radical Scavenging method was used for antioxidant activity test. Qualitative phytochemical analysis of leaf extracts of *Clausena anisata* (Willd.) Hook showed the presence of alkaloids, flavanoids, carbohydrates, saponnin, tannin, coumarin, proteins and aminoacids. Highest Scavenging was observed with leaf extracts of ethylacetate+chloroform (74.1%), acetone (74%), aqueous (70.8%) followed by hexane (67.1%) and alcohol (55.1%) at 100mg/ml. Antibacterial activity of ethylacetate+Chloroform extract was higher for cornybacterium and Proteus with 43mm zone of inhibition and for S.epidermis with 32mm zone of inhibition. It was found that the percentage increase of the rate of uptake of glucose into yeast cells was linear in different glucose concentrations used. The aqueous and alcohol leaf extracts of *Clausena anisata* (Willd.) Hook showed the maximum increase in 10mM glucose concentration i.e. 95% and 95.3% at 2000 µg/ml. The alcohol, aqueous and acetone extracts of *Clausena anisata* (Willd.) Hook showed appreciable (>20%) enzyme inhibitory activity against human urinary alpha amylase followed by hexane (3.4%) and ethylacetate+chloroform extracts (8.1%).

Keywords: *Clausena anisata*, Antioxidant, Antidiabetic, Antibacterial

INTRODUCTION

The pancreas is a glandular organ in the digestive system and endocrine system of vertebrates. It is an endocrine gland producing several important hormones, including insulin, glucagon, somatostatin, and pancreatic polypeptide which circulate in the blood. The pancreas is also a digestive organ, secreting pancreatic juice containing digestive enzymes that assist digestion and absorption of nutrients in the small intestine[1]. Diabetes is a disorder of metabolism-the way the body uses digested food for energy. The digestive tract breaks down carbohydrates-sugars and starches found in many foods-into glucose, a form of sugar that enters the bloodstream. With the help of the hormone insulin, cells throughout the body absorb glucose and use it for energy. The two main types of Diabetes are Type 1 Diabetes and Type 2 Diabetes. A third type, gestational Diabetes, develops only during pregnancy [2]. In light of the fact that the majority of drugs in clinical use today are enzyme inhibitors, it is reasonable to expect that enzyme inhibitors could also be useful in the treatment of Diabetes mellitus. Indeed, research conducted during the last few decades has identified a number of carbohydrate metabolizing enzymes as possible targets for antidiabetic treatment. These carbohydrate metabolizing enzymes include the digestive enzymes α-amylase and α-glucosidase and gluconeogenic enzymes[3], in particular α-amylase that are was studied.

The antioxidant activity of these two essential oils may be related to their hydrogen-donating ability. The lower potential and easier formation of radicals indicate the higher hydrogen-donating ability of antioxidants[4]. The antioxidant activity of antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [5].

Clausena anisata (Willd.) Hook has got a lot of antibacterial properties. Its leaves are crushed and applied to wounds infested with maggots for treatment [6]. The reported pharmacological activities are justified by the results of antimicrobial screening together with the phytochemistry of the plant. Glucose transport in Saccharomyces cerevisiae depends on carrier-mediated, nonconcentrative facilitated diffusion; the same carrier is used for glucose, fructose, and mannose[7]. Concentrative uptake by proton symport, known to exist in other yeast , has not been reported for S.cerevisiae. In 1942 Jacques Monod introduced his microbial growth model that prompted quantitative studies of microbial metabolism. This motivated a wealth of mathematical models describing the growth of budding yeast S. cerevisiae on the key carbohydrate glucose [8].

Clausena anisata (Willd.) Hook is a large evergreen strong-smelling herb naturalized in many parts of India. All parts of this plant have been used traditionally for several ailments throughout the world. Several tannins and coumarins isolated from this plant are known to exert diverse biological activities including antidiabetic properties.

MATERIALS AND METHODS

Collection of plant material

Whole plants of *Clausena anisata* (Willd.) Hook was collected in the month of November from Viralimalai (Tiruchirappalli District). The collected plant materials was powdered and further used for solvent extraction.

Methods of extraction

The leaves were collected and then the material was ground finely in the Pestle and Mortar. The ground material was weighed and approximately 5g of leaf material was extracted successively using solvents of varying polarity by maceration method. After extraction the extract was incubated for three days at room temperature. The concentrated extracts were evaporated to dryness and weighed.

Phytochemical studies on *Clausena anisata* (Willd.) Hook

The extracts obtained after successive solvent extraction were qualitatively tested for the presence of various phytochemicals. Different phytochemicals have been isolated from various morphological parts of the plant [9]. C. anisata (Willd.) Hook has been widely reported to contain large amounts of coumarins, (especially scopoletin, chalepin, helietin, osthole, coumarrayin, xanthoxyletin, heliettin, imperatorin, furanocoumarin derivatives) and volatile

oil containing phenylpropanoids[10],[11]. Phyto chemical screening of the crude leaf extract was carried out employing standard procedures and tests to reveal the presence of chemical constituents such as alkaloids, flavanoids, tannins, terpenes, saponins, anthraquinones, reducing sugars, cardiac glycosides among others.

Phytochemical analysis

The different extract was subjected to different chemical test for the detection of different phytoconstituents using standard procedure [12].

Antioxidant activity of *Clausena anisata* (Willd.) Hook

DPPH radical Scavenging activity

The antioxidant activity of *Clausena anisata* aqueous, leaf extract and the standard antioxidant ascorbic acid was assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical activity according to the method described by [13] C.anisata aqueous extract with different concentrations (100,200,300,400,500 mg/ml) were prepared in aqueous. Ascorbic acid was used as standard in 1mg/ml concentration. The scavenging activity against DPPH was calculated using the following equation: Scavenging activity (%) = [(A-B)/A] X 100, where A was the absorbance of control (DPPH solution without the sample), B was the absorbance of DPPH solution in the presence of the sample (extract/ascorbic acid).

Antidiabetic activity of *Clausena anisata* (Willd.) Hook

Alpha amylase

Inhibition assay for the enzyme α-amylase was performed in accordance with the standard, chromogenic In vitro inhibition procedures modified by Benfeld [14]. Porcine pancreatic amylase (Sigma-Aldrich) or urine amylase was dissolved in ice-cold 20 mM phosphate buffer (pH 6.7) containing 6.7 mM sodium chloride to give a concentration of 5 unit/ml. Seven duplicate test tubes including the blank and control were prepared. In each test tube 250 µl of the enzyme preparation was mixed with 100 µl of each of the leaf extracts of C. anisata (Willd.) Hook except the blank (hexane, acetone, alcohol and water, 2 mg/ml). The mixtures were stirred in a vortex and pre-incubated in a water bath at 37 °C for 20 minutes. After incubation, 250 µl of the substrate preparation (0.5 % w/v starch, in 20 mM phosphate buffer, pH 6.7) was transferred into each test tube to start the reaction. The mixture was vortexed and then incubated at 37 °C for 15 minutes. Two millilitres (2 ml) of DNS colour reagent was added and the mixture was stirred in a vortex and boiled in a water bath at 100°C for 10 minutes, thereafter the mixture was cooled down in a running tap water and the absorbance was read at 540 nm using a spectrophotometer.

Inhibition rates were calculated as percentage controls using the formula:

% inhibition = 100 – % reaction

(where % reaction = mean product in sample/ mean product in control x 100)

Glucose uptake in yeast cells

The commercial baker's yeast in distilled water was subjected to repeated centrifugation (3000Xg,5min) until clear supernant fluids were obtained and a 10% (v/v) of the suspension was prepared in distilled water. Various concentrations of plant extracts (50-2000µg/mL) were added to 1mL of glucose solution (5,10, 25mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100µL of yeast suspension followed by vortexing and further incubation at 37°C for 60min. After 60 minutes, the tubes were centrifuged (2,500Xg, 5 min) and amount of glucose was estimated in the supernatant. Metromidazole was used as standard drug [15].

The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

Increase in glucose uptake (%) = Abs sample – Abs control X 100 / Abs sample.

Where, Abs control is the absorbance of the control reaction.

Abs sample is the absorbance of the test sample.

Anti-Bacterial acitivity test

Micro-organisms used

Gram positive - C.perfringens, Cornybacter, E.feacalis, S.aureus.

Gram negative- E.coli, K.pneumonia, P.aeruginosa, Proteus species.

These Gram positive and Gram negative organisms were inoculated into the 2ml nutrient broth tubes separately and incubated over night at 37°C. In the next day, the four nutrient plates were prepared for the respective organisms.

Agar well diffusion method

The agar media was poured into petri dishes where 0.1 ml of micro-organism had been seeded. The mixture was swirled and the agar was left to solidify. Lids of the petridishes were kept closed as much as possible to prevent contamination. Using a borer of diameter 4mm four equidistant wells were punched with flaming. Plant extracts were then introduced and the plates were incubated within 15 minutes after applying the discs since the test is standardized under conditions where diffusion of the antibiotic and bacterial growth commence approximately at the same time. The diameter of the zones of growth inhibition around each well were measured and recorded to the nearest mm using a ruler [16].

Disc diffusion method

The antibacterial activity of the leaves extracts were tested against the selected bacterial strains the 20ml of sterilized nutrient agar medium are poured into each sterile Petri plate and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cork borer 150µL of each ethanol and aqueous. Plant extracts were transferred into separate well after these plants were incubated at 37°C for 24-48hours. After incubation period the result were observed and measure the diameter of incubation zone around the each well. The standard antibiotics ciprofloxacin as a (positive) and respective alcohol as a (negative) solvent was used as control [16].

STATISTICAL ANALYSIS

The data was expressed as mean ± standard error mean (S.E.M). The significance of differences among the group was assessed using one way and multiple way analyses of variance (ANOVA).

RESULTS AND DISCUSSION

Plant Collection

The Collected plant was identified by Dr. S. John Britto, Director The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Campus) Tiruchirappalli-620 002 and authenticated as *Clausena anisata* (Willd.) Hook by Specimen No: LB NSD 001. The collected plant specimen was pressed on newspaper and left for 24 hours. The paper was changed everyday till complete drying. The dried specimen was mounted on the standard size of herbarium sheet. The specimen was fixed into herbarium sheet and labeled (Fig. 1). The leaves of *Clausena anisata* (Willd.) Hook were shade dried at room temperature.

Fig 1: Herbarium of *Clausena anisata* (Willd.) Hook

Percentage yield of leaf extracts of C.anisata (Willd.) Hook

Two hundred and fifty grams (250 g) of dried and powdered leaves of C.anisata (Willd.) Hook was sequentially extracted with solvent of decreasing polarity (water, alcohol, acetone, ethylacetate +chloroform and hexane). The percentage yields of extracts obtained with these solvents are shown in (Fig. 2).

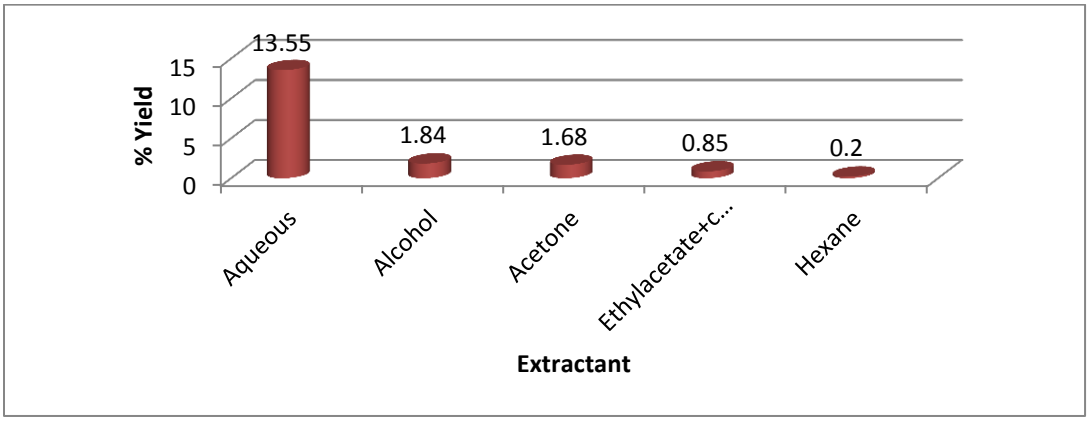

Fig 2: Percentage yield of C.anisata (Willd.) Hook leaf extracts

Table 1 indicate the different solvents used in the extraction, namely hexane, ethylacetate+ chloroform, acetone, alcohol and water. The mass and the percentage yield of each solvent extract are also shown. The mass of each extract was obtained after evaporation of the solvent (extractant). The highest percentage yield in mass was observed on the water extracts of C.anisata (Willd.) Hook, followed by aqueous, alcohol, acetone, ethylacetate+ chloroform and hexane. Extraction with hexane produced the lowest percentage yield. % yield of the extract is the total dry mass after evaporation of the solvent (e.g. hexane) and it was calculated as follows:

% Yield=mass of dry extract/total mass dry powdered sample X 100

Water, alcohol, acetone, ethylacetate +chloroform and hexane extracts of C.anisata (Willd.) Hook obtained by sequential extraction were screened for further studies.

Table 1: Mass and percentage yield of extractant

Extractant	Mass of extract of C.anisata (Willd.) Hook leaves (g)	Percentage yield of extractant (%)
Hexane	0.52	0.20
Ethylacetate+ chloroform	2.13	0.85
Acetone	4.2	1.68
Alcohol	4.6	1.84
Water	33.89	13.55

Phytochemical Screening

The Phytochemical screening of the aqueous, alcohol, acetone, ethylacetate +chloroform and hexane extract of *Clausena anisata* (Willd.) Hook were carried out. The results were tabulated from Table 2. The extracts obtained from the maceration of the leaf were utilized in antioxidant, antidiabetic, alpha-amylase and antibacterial activity test. Fig. 3-Fig. 7 shows the presence of phytoconstituents in the aqueous, alcohol, acetone, ethylacetate+chloroform and hexane extract of *Clausena anisata* (Willd.) Hook.

Table 2: Phytochemical Screening of leaf of *Clausena anisata*

S.NO	Name of the test	Methanol extract	Acetone extract	Aqueous extract	Hexane extract	Ethylacetate chloroform extract
1.	Alkaloid	+	+	+	+	+
2.	Flavonoids	+	_	+	+	_
3.	CHO and glycosides	+	_	_	_	+
4.	Saponification	+	_	+	_	_
5.	Protein and aminoacid	_	+	_	+	+
6.	Tannins	+	_	+	_	_
7.	Gum and mucilage	_	_	_	_	_
8.	Anthocyanin	_	_	_	_	_
9.	Leucoanthocyanin	_	_	_	_	_
10.	Coumarin	_	_	+	_	_

Positive-Presence; Negative- Absence

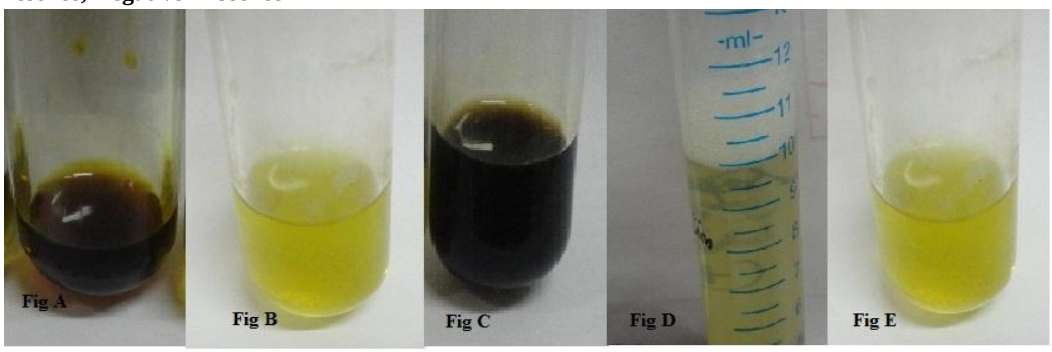

Fig 3: Phytochemical analysis shows the presence of Phytoconstituents in the aqueous extract of *Clausena anisata* (Willd.) Hook. a) Test for alkaloid (Wagner's test); b) Test for alkaloid (Hager's test); c) Test for flavanoids (Ferric Chloride test); d) Test for Saponins; e) Test for coumarins.

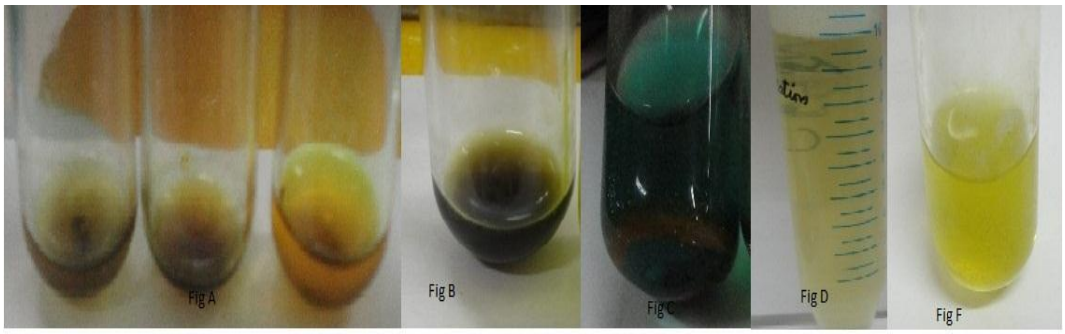

Fig 4: Phytochemical analysis shows the presence of Phytoconstituents in the alcohol extract of *Clausena anisata* (Willd.) Hook. a) Test for alkaloid (Mayer's test, Wagner's test, Hager's test); b) Test for flavanoids (Ferric Chloride test); c)Test for Carbohydrates (Benedict's test); d)Test for Saponins; e)Test for coumarins

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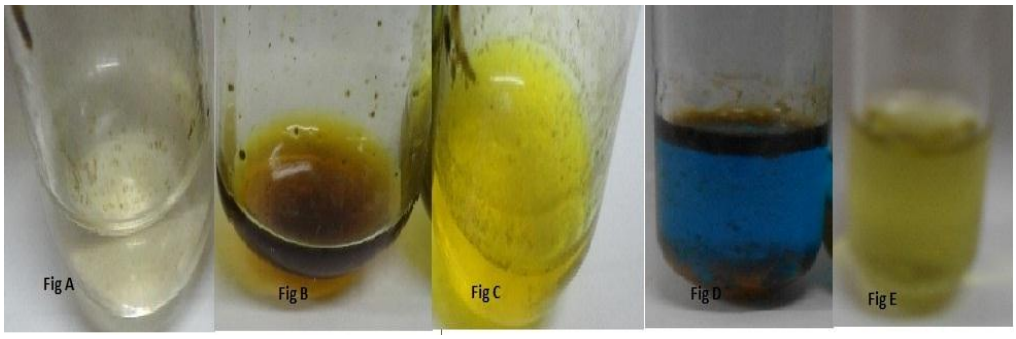


Fig 5: Phytochemical analysis shows the presence of Phytoconstituents in the acetone extract of *Clausena anisata* (Willd.) Hook. a) Test for alkaloid (Mayer's test); b) Test for alkaloid (Wagner's test); c) Test for alkaloid (Hager's test); d) Test for carbohydrates (Fehling's test); e) Test for proteins and aminoacids (Xanthoproetic test).

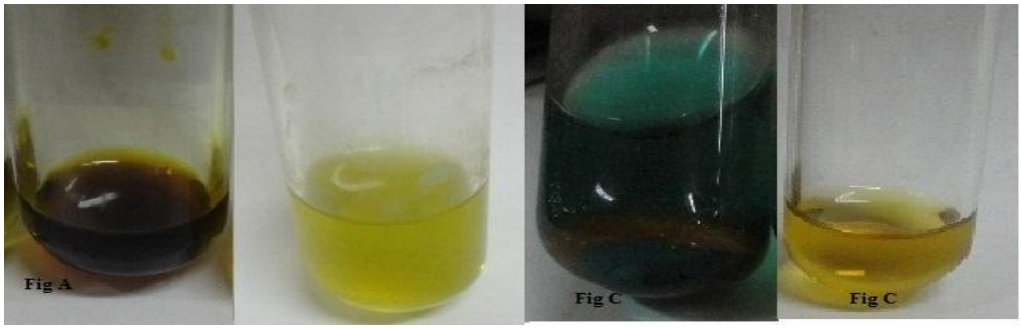


Fig 6: Phytochemical analysis shows the presence of Phytoconstituents in the ethylacetate+chloroform extract of *Clausena anisata* (Willd.) Hook. a) Test for alkaloid (Wagner's test); b)Test for alkaloid (Hager's test); c) Test for carbohydrates (Fehling's test); d) Test for proteins and aminoacids (Xanthoproetic test).

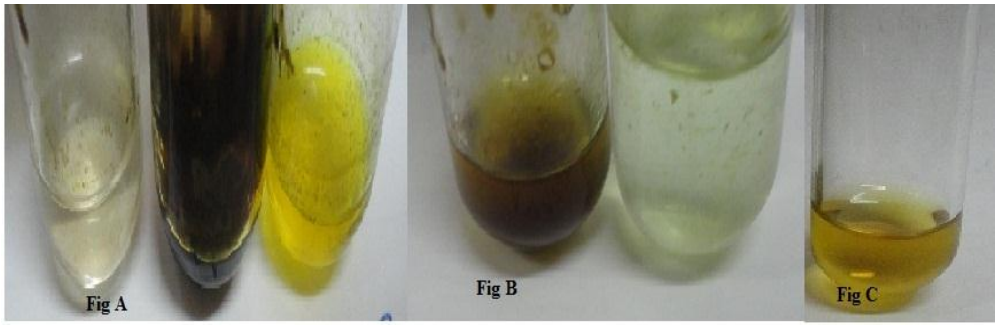


Fig 7: Phytochemical analysis shows the presence of Phytoconstituents in the hexane extract of *Clausena anisata*(Willd.) Hook. a) Test for alkaloid (Mayer's test, Wagner's test, Hager's test); b) Test for flavanoids (Ferric Chloride and NaOH test); c) Test for proteins and aminoacids (Xanthoproetic test).

Phytochemical screening is of para-amount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth. The identification of secondary metabolites is the prime or prerequisite step in this investigation. The phytochemical character of the leaf of *Clausena anisata* (Willd.) Hook were investigated qualitatively. The phytochemical screening revealed the presence of alkaloid in all the five solvent (leaf) extracts of *Clausena anisata* (Willd.) Hook.

The phytochemical screening revealed the presence of saponin, tannin, coumarin in the aqueous and alcohol extracts of *Clausena anisata* (Willd.) Hook. The phytochemical screening revealed the presence

of protein and aminoacids in the acetone, ethylacetate+ chloroform and hexane extracts of *Clausena anisata* (Willd.) Hook. The phytochemical screening revealed the presence of flavanoids, CHO and glycosides in the aqueous and ethylacetate +chloroform extracts of *Clausena anisata* (Willd.) Hook.

Fluorescence analysis of *Clausena anisata* leaf

Clausena anisata (Willd.) Hook leaf powder on treatment with different chemical reagents, was observed in visible light were represented in Table 3. Powder of leaf appeared to be green colour. On treatment with conc.H_2SO_4 and iodine it gives brown colour. When powders of *Clausena anisata* (Willd.) Hook leaves treated with ammonium hydroxide, chloroform, picric acid and conc.nitric acid it gives yellow colour. On treatment with ferric chloride, and acetone it gives blackish green colour. When powders of *Clausena anisata* (Willd.) Hook leaves treated with ammonia, HCl and isoamyl alcohol it gives green colour. Finally *Clausena anisata* (Willd.) Hook leaves treated with NaOH it gives golden yellow. Many phyto drugs when suitably illuminated emit light of different wave length or colour from sthat which falls on them. The fluorescence analysis of drug extract helps to identify the drug with specific fluorescent colours and also to find out the fluorescent impurities.

Table 3: Fluroescence analysis of *Clausena anisata* leaf (Willd.) Hook

S.No	Particulars of the Treatment	Under ordinary light
1.	Powder + Ferric chloride	Blackish green
2.	Powder + Conc. $H_2 SO_4$	Brown
3.	Powder + acetone	Blackish green
4.	Powder + iodine	Brown
5.	Powder + NaOH	Golden yellow
6.	Powder + conc. Nitric acid	Yellow
7.	Powder + HCL + NH_3	Green
8.	Powder + isoamyl alcohol	Green
9.	Powder + NaOH	Yellow
10.	Powder + chloroform + ammonia	Yellow

In vitro antioxidant activity

The free radicals are chemical species which contains one or more unpaired electrons. They are highly unstable and cause damage to other molecules by extracting electrons from them in order to attain stability. Radical scavenging activities are very important due to the deleterious role of free radicals in biological systems. In vitro antioxidant activity of alcohol, acetone, and hexane, extracts of *Clausena anisata* (Willd.) Hook leaves was analysed by total antioxidant capacity.

Total antioxidant assay

Total antioxidant capacity of the aqueous, alcohol, acetone, ethylacetate+chloroform and hexane extract of *Clausena anisata* (Willd.) Hook were evaluated by the DPPH method. The absorbance value at 517nm and % inhibition was represented in Table 4 and Table 5. The comparative analysis of Total antioxidant activity between the aqueous, alcohol, acetone, ethylacetate+chloroform and hexane extract of *Clausena anisata* (Willd.) Hook were represented in the Fig. 8. DPPH test is based upon the ability of DPPH, a stable free radical, to decolourize from purple in the presence of antioxidants. It is a direct and dependable method for determining the radical scavenging action. Ascorbic acid was chosen as the standard antioxidant for this test. The DPPH radical contains on odd electron, which is responsible for absorbance at 517nm and also for a noticeable deep purple colour, when DPPH accepts an electron donated by an antioxidant compound the DPPH becomes colourless, which is quantitatively measured from the changes in absorbance. Highest Scavenging was observed with leaf extracts of ethylacetate +chloroform (74.1%), acetone (74%), aqueous

(70.8%), followed by hexane (67.1) and alcohol (55.1). The scavenging activity of DPPH radical was found to rise with increasing concentration of extracts. Additionally it has been determined that the antioxidant effect of plant product is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols and tannins. The present result suggest that all the tested plant extracts have moderate to potent antioxidant activity. It becomes very difficult to describe the antioxidant properties selectively to any one group of constituents without further studies it is impossible. Thus further thorough investigations are necessary.

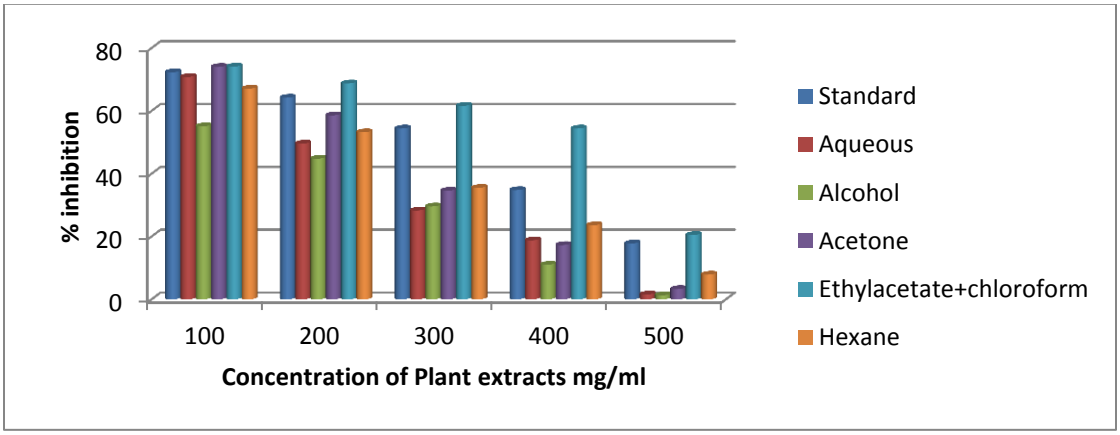
Table 4: UV Absorbance of *Clausena anisata* (Willd.) Hook in different extract and Ascorbic acid for antioxidant activity

S.No	Concentration (mg/ml) of extracts/Ascorbic acid	Absorbance of extracts/Ascorbic acid at 517 nm					
		Aqueous	Alcohol	Acetone	Ethylacetate Chloroform	Hexane	Ascorbic acid (Std)
1.	100	0.37	0.65	0.39	0.29	0.50	0.31
2.	200	0.64	0.80	0.62	0.35	0.71	0.40
3.	300	0.91	1.02	0.98	0.43	0.98	0.51
4.	400	1.03	1.29	1.24	0.51	1.16	0.73
5.	500	1.25	1.43	1.45	0.89	1.40	0.92

Table 5: Percentage inhibition of DPPH free radical by *Clausena anisata* (Willd.) Hook extracts/ascorbic acid at 517nm

S.No	Concentration (mg/ml) of extracts/Ascorbic acid	% inhibition of DPPH free radical by extracts/ascorbic acid at 517 nm					
		Aqueous	Alcohol	Acetone	Ethylacetate+ Chloroform	Hexane	Ascorbic acid (Std)
1.	100	70.8	55.1	74	74.1	67.1	72.3
2.	200	49.6	44.8	58.6	68.75	53.2	64.2
3.	300	28.3	29.6	34.6	61.6	35.5	54.4
4.	400	18.8	11.03	17.3	54.4	23.6	34.8
5.	500	1.57	1.3	3.3	20.5	7.89	17.8

Fig 8: DPPH Radical Scavenging activity of *Clausena anisata* (Willd.) Hook leaf extracts.



In vitro antidiabetic activity

Regulation of glucose level in the blood of the diabetic patient can prevent the various complications associated with the disease. The maintenance of plasma glucose concentration for a long term under a

variety of dietary conditions is one of the most important and closely regulated processes observed in the mammalian species.

Alpha amylase Inhibitory assay

Alpha amylase is an enzyme that hydrolyses alpha-bonds of large alpha linked polysaccharide such as glycogen and starch to yield glucose and maltose. Alpha amylase inhibitors bind to alpha bond of polysaccharide and prevent break down of polysaccharide in monosaccharide and disaccharide. As the result shows (Table 6 and Fig. 9). The alcohol, aqueous and acetone extracts of *Clausena anisata* (Willd.) Hook showed appreciable (>20%) enzyme inhibitory activity against human urinary alpha amylase followed by hexane (13.4%) and ethylacetate+chloroform extracts (8.1%), compared to the standard drug.

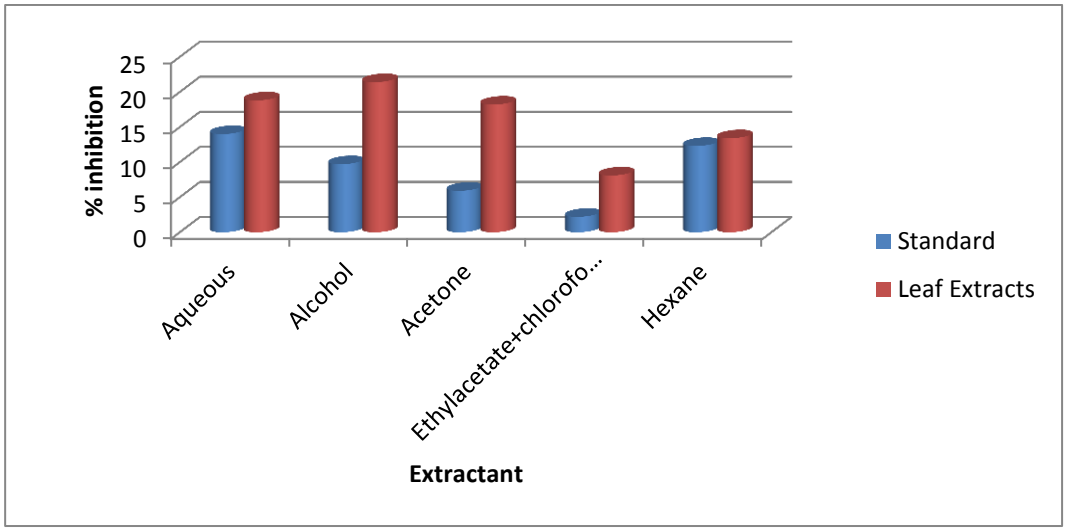


Fig 9: Enzyme inhibitory effects of *Clausena anisata* (Willd.) Hook leaf extracts.

Glucose uptake in yeast cell

The In vitro antidiabetic activity of the leaves of *Clausena anisata* (Willd.) Hook possess good anti diabetic activity (Table 7- Table 9). In Yeast (Saccharomyces cerevisiae) glucose transport takes place through facilitated diffusion. After the treatment of the yeast cells with the extracts of *Clausena anisata* (Willd.) Hook the glucose uptake was found to increase in a dose dependent manner. The rate of glucose transport across cell membrane in yeast cells system is presented in Fig. 10 – Fig. 12. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. The rate of uptake of glucose into yeast cells was linear in all the three glucose concentration. The aqueous and alcohol extract exhibited significantly higher activity than other extracts in all concentrations. However the highest uptake of glucose was seen in 10mM glucose concentration, i.e. 95% and 95.3% at 2000µg/ml.

The mechanism of glucose transport across the yeast cell membrane has been receiving attention as In vitro screening method for hypoglycaemic effect of various compounds/medicinal plants. Recent studies on the transport of non metabolizable sugars and certain metabolizable glycosides suggest that sugar transport across the yeast cell membrane is mediated by stereo specific membrane carriers. It is reported that in yeast cells (Saccharomyces cerevisiae) glucose transport I yeast is by a facilitated diffusion process. Facilitated carriers are specific carriers that transport solutes down the concentration gradient. This means that effective transport is only attained if there is removal of intracellular glucose.

Table 7: % inhibition of Glucose uptake by yeast cells at 5mM Glucose Concentration.

Glucose 5mM											
Blank	**Conc µg/ml**	**Standard (Metromidazole)**		**Aqueous**		**Alcohol**		**Acetone**		**Hexane**	
		Abs	% inhibition	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition
	50	0.10	70	0.07	42.8	0.07	57.1	0.08	37.5	0.08	37.5
	500	0.24	87.5	0.18	77.7	0.14	78.5	0.16	68.7	0.12	58.3
0.05	1000	0.40	92.5	0.34	88.2	0.31	90.3	0.42	88	0.24	79.1
	1500	0.54	94.4	0.50	92	0.45	93.3	0.60	91.6	0.39	87.1
	2000	0.59	94.9	0.64	93.7	0.65	95.3	0.86	94.1	0.59	91.5

Fig 10: Absorbance of *Clausena anisata*(Willd.) Hook leaf extracts in 5mM glucose concentration

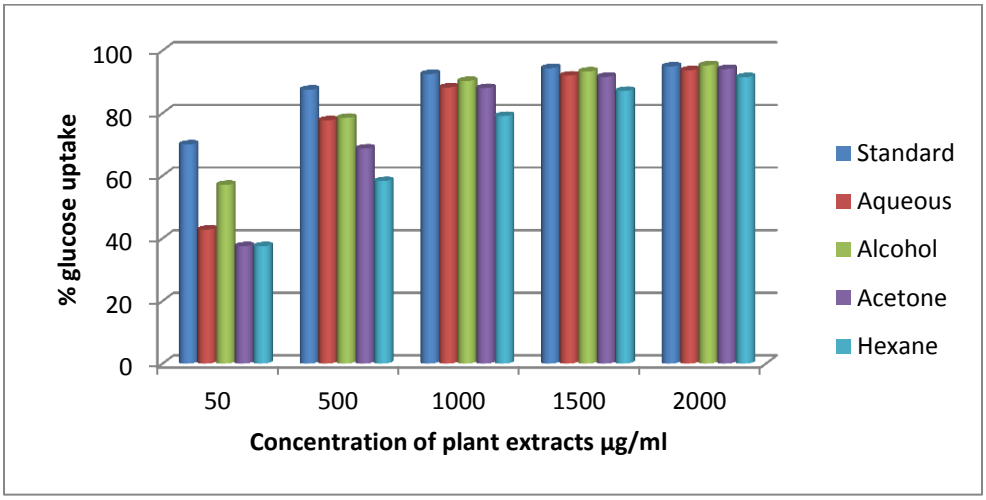


Table 8: % inhibition of glucose uptake in 10mM glucose concentrations

Glucose 10mM										
Conc µg/ml	**Standard (Metromidazole)**		**Aqueous**		**Alcohol**		**Acetone**		**Hexane**	
	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition
50	0.09	66.6	0.19	78.9	0.05	40	0.11	54.5	0.18	72.2
500	0.22	86.3	0.32	87.5	0.12	75	0.22	77.2	0.25	80
1000	0.45	93.3	0.46	91.3	0.34	91.1	0.39	87.1	0.40	87.5
1500	0.55	94.5	0.65	93.8	0.48	93.7	0.54	90.7	0.51	90.1
2000	0.59	94.9	0.80	95	0.64	95.3	0.68	92.6	0.71	92.9

Fig 11: Absorbance of *Clausena anisata* (Willd.) Hook leaf extracts in 10mM glucose concentrations

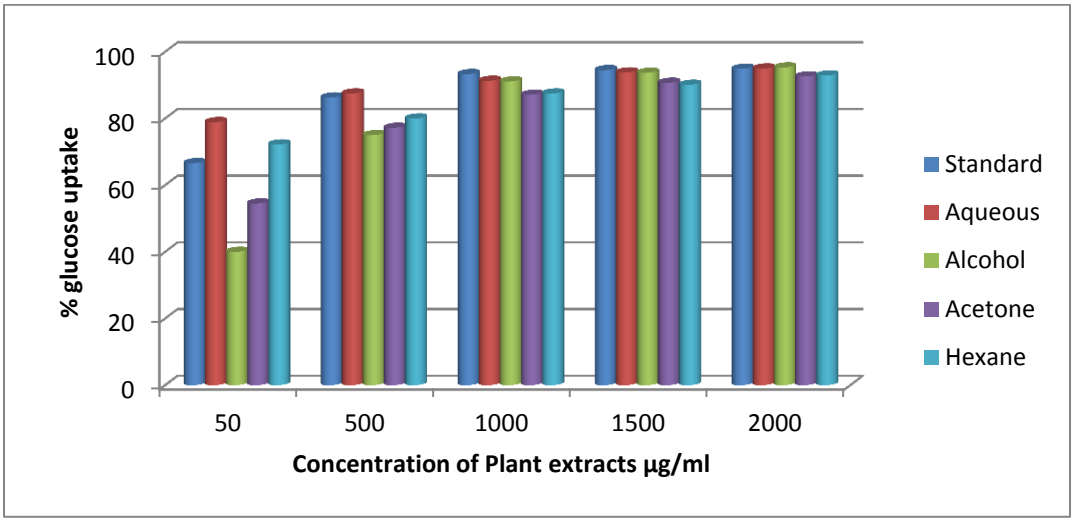
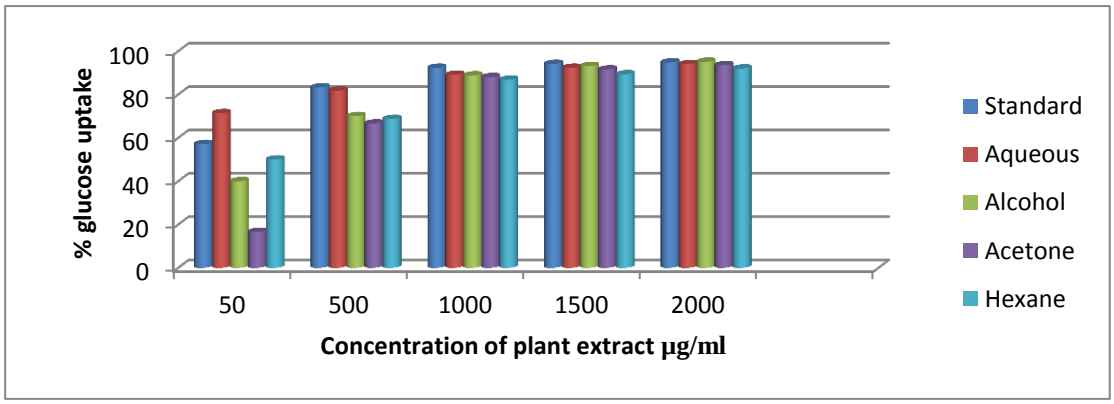


Table 9: % inhibition of glucose uptake in 25mM glucose concentrations

Glucose 25mM										
Conc µg/ml	Standard (Metromidazole)		Aqueous		Alcohol		Acetone		Hexane	
	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition	Abs	% inhibition
50	0.07	57.1	0.14	71.4	0.05	40	0.06	16.6	0.10	50
500	0.18	83.3	0.22	81.8	0.10	70	0.15	66.6	0.16	68.7
1000	0.39	92.3	0.37	89.1	0.27	88.8	0.42	88	0.38	86.8
1500	0.51	94.1	0.52	92.3	0.44	93.1	0.59	91.5	0.47	89.3
2000	0.58	94.8	0.67	94	0.62	95.1	0.78	93.5	0.63	92

Fig 12: Absorbance of *Clausena anisata* (Willd.) Hook leaf extracts in 25mM glucose concentrations



In Vitro Antibacterial activity

Antibacterial activity of leaf extracts of *Clausena anisata* (Willd.) Hook were evaluated In vitro against Gram positive and Gram negative organisms, which are known to cause infections in human. All extracts studied in this work showed antibacterial activity

against the test micro-organisms (Table 10 and Fig. 13). Result of antibacterial test shows that ethylacetate+chloroform extract have the greater zone of inhibition (43mm) for Cornybacterium and Proteus, followed by S.epidermis (32mm), S.aureus (10mm), E.feacalis (9mm). Acetone extract of *Clausena anisata* have the Zone of inhibition of (19mm) for K.pnemoniae followed by S.aureus (14mm), Proteus (11.6mm), C.perfringers (9mm) and E.coli (3mm). Hexane extracts of *Clausena anisata* (Willd.) Hook have the Zone of inhibition of (6mm) for E.coli and (5.2mm) for K.pneumoniae where as alcoholic extract of *Clausena anisata* (Willd.) Hook have the Zone of inhibition of (5mm) for E.coli and (4mm) for C.perfringers and it has least antibacterial activity.

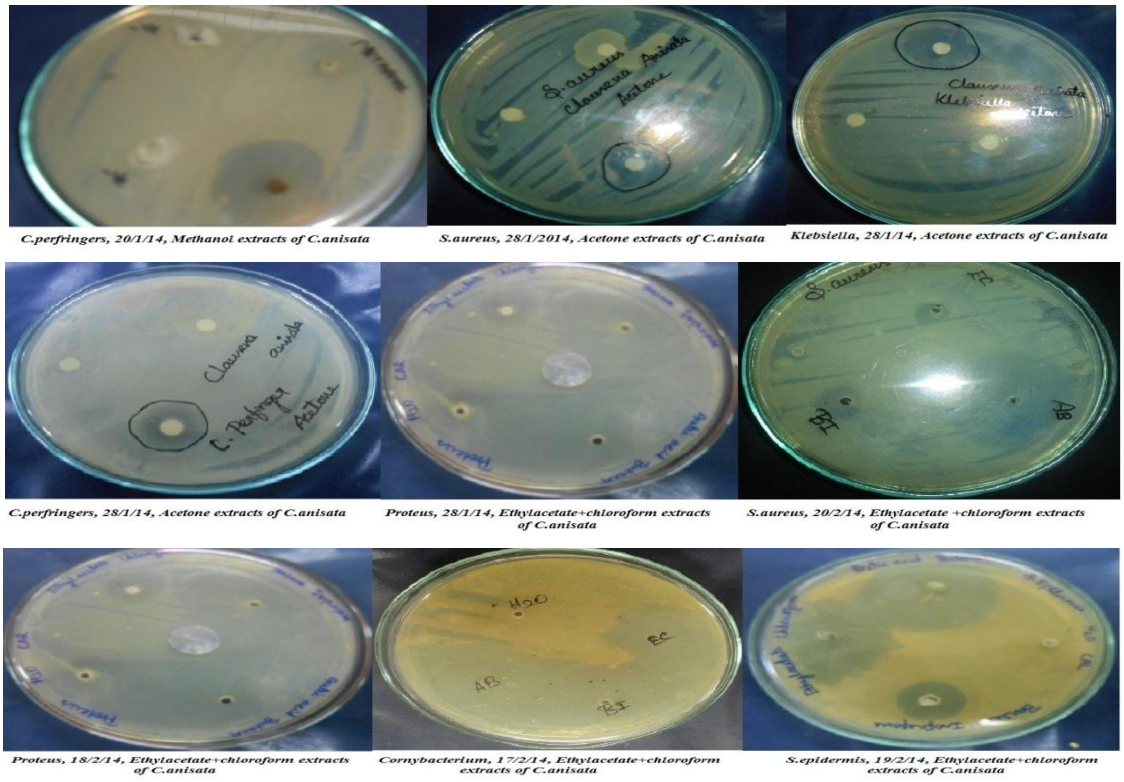
Table 10: Effect of Antibacterial activity on *Clausena anisata* (Willd.) Hook leaf

S.No	Test Organisms	Alcohol	Acetone	Hexane	Ethylacetate +Chloroform
1.	C.perfingers	+	+	-	-
2.	Cornybacterium species	-	-	-	+
3.	E.coli	+	+	+	-
4.	E.feacalis	-	-	-	+
5.	K.pneumoniae	-	+	+	-
6.	Proteus species	-	+	-	+
7.	S.aureus	-	+	-	+
8.	S.epidermis	-	-	-	+

(+) Presence of zone of inhibition

(-) Absence of zone of inhibition

Fig 13: Antibacterial activity of the leaf extracts of *Clausena anisata* (Willd.) Hook



C.perfringers, 20/1/14, Methanol extracts of C.anisata

S.aureus, 28/1/2014, Acetone extracts of C.anisata

Klebsiella, 28/1/14, Acetone extracts of C.anisata

C.perfringers, 28/1/14, Acetone extracts of C.anisata

Proteus, 28/1/14, Ethylacetate+chloroform extracts of C.anisata

S.aureus, 20/2/14, Ethylacetate +chloroform extracts of C.anisata

Proteus, 18/2/14, Ethylacetate+chloroform extracts of C.anisata

Cornybacterium, 17/2/14, Ethylacetate+chloroform extracts of C.anisata

S.epidermis, 19/2/14, Ethylacetate+chloroform extracts of C.anisata

CONCLUSION

Several plants are currently being investigated to know their medicinal properties. The above conducted In vitro studies depict that the presence of the phytochemicals in these plant might be the reason for antioxidants, antidiabetic and antibacterial activity. The plant *Clausena anisata* (Willd.) Hook may essentially contain herbal bioactive compounds which require further structured elucidation and characterization methodologies to identify the bioactive constituents. Further research is underway to evaluate the effect of the leaf extracts in In vivo, to purify and to characterize the compounds from the plants. These plant will be explored further for their biopharmaceutical and industrial uses.

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