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In vitro evaluation of antidiabetic and anti-inflammatory activities of polyphenolic-rich fraction from *Naval Kottai Chooranam* (*Eugenia Jambolan*)

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Abstract
This study was focused on the phytochemical composition and biological activities of *Naval kottai chooranam* (*Eugenia jambolan*) polyphenolic-rich extracts. The phytochemical composition and thin layer chromatography analysis showed that variety of phytochemical. *Naval kottai chooranam* (*Eugenia jambolan*) polyphenol rich extract had the highest α-amylase inhibitory activity (EC_{50} = 64.23 µg/mL) and α-glucosidase inhibitory activity (EC_{50} = 72.31µg/mL). However, polyphenolic-rich extracts presented a significant α-glucosidase inhibitory activity. Furthermore, the protease and lipoxygenase inhibition of polyphenolic-rich extracts also proved to be stronger (EC_{50} = 83.32 µg/mL), but there was moderate or low lipoxygenase inhibition. The experimental data suggest that both extracts are promising candidates for the development of natural antidiabetic and anti-inflammatory food supplements.

Keywords: antidiabetic and anti-inflammatory; *Naval kottai chooranam*

Introduction

Plants have played a significant role in providing the human race with remedies. Plants play important roles in human life not only as suppliers of oxygen but also as a fundamental resource to sustain the human race on this earthly plane. Plants also play a major role in our nutrition by converting energy from the sun during photosynthesis. In addition, plants have been used extensively in traditional medicine since time immemorial. Medicinal plants have been stated to comprise about 8000 species and account for approximately 50% of all the higher flowering plant species of India. In other words, there are about 400 families of the flowering plants; at least 315 are represented by India (Solomon, 2019) [15]. Medicinal properties of few such plants have been reported but a good number of plants still used by local folklore are yet to be explored. Ayurveda, Siddha and Unani systems of medicine provide good base for scientific exploration of medicinally important molecules from nature. Antioxidants play an important role in inhibiting and scavenging free radicals, thus providing protection to humans against infections and degenerative diseases; however, recent concern has been paramount regarding the potential detrimental side effects of synthetic additives in humans (Rajkumar *et al*., 2010).

Considering the fact that diabetes is regarded as a chronic metabolic disease, numerous antidiabetic therapies with conventional drugs are often not a single-dose program as most drugs require frequent injections, sometimes for the entire life of the diabetic patient. However, many of these conventional drugs have been reported for their inefficiency with prominent adverse side effects (Gupta *et al*., 2016) [3]. These limitations have largely prompted the exploration of management strategies involving the use of medicinal plants reported to be cost-effective antidiabetic agents with fewer reported side effects (Atanasov *et al*., 2015) [1]. However, the majority of these traditional plants have not been scientifically validated for their efficacy in the treatment of diabetes. *Naval kottai chooranam* (*Eugenia jambolan*) is among such plants traditionally used among local healers in the South India. Antioxidants are also being investigated as possible treatments for neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Amyotrophic Lateral Sclerosis and as a way to prevent noise induced hearing loss. People take antioxidant supplements directly from fresh fruits and vegetables. Fruits and vegetables contain a large amount of flavonoids and antioxidant supplements that contribute to protection against different types of cancers and cardiovascular health problems (Hamid *et al*., 2010) [4].

Flavonoids, low molecular weight polyphenols of plant origin are a group of naturally occurring compounds. These are widely distributed in the human food supply through fruits and vegetables and are considered to bear potential anticarcinogenic effects. These are believed to be good scavengers of free radicals. Around 28 naturally occurring and synthetic flavonoids have been suggested as novel anti leukamic compounds. Besides, flavonoids have also been reported to exert multiple biological effects including anti-inflammatory anti allergic, antiviral and anticancer activity (Sharoni *et al*., 2000) [14]. Lycopene It is widely accepted fact that diet changes are powerful tool for cancer prevention and inhibition of cancer progression. It has been found that lycopene can significantly reduce the risk of prostate cancer in men. Not only this, it is helpful in preventing cancer of pancreas, colon, rectum, oesophagus, oral cavity, large bowel, ovaries, cervice and mouth. Lycopenes have a specific role in preventing heart disease and protect the skin against sun damage (Samaranayaka and Li-Chan, 2011) [13]. *Syzygium cumini* L. (synonym: *Syzygium jambolana*, *Eugenia jambolana*, *Eugenia cumini*) belong to family Myrtaceae, commonly known as Indian blackberry or Jamun. *S. cumini*, an evergreen tropical tree, is

native to Indian subcontinent and naturalized in America, Africa, and Australia (Aqil *et al*., 2015). The oblong berries having deep purple to violet color with pinkish pulp are widely consumed as fruit. In addition to its nutraceutical value, fruits are used in traditional medicine for treatment of various diseases. *In vitro* studies of the fruits extracts showed antiinflammatory, antioxidant, antidiabetic and antimutagenic activity, and also act as detoxifier (Ramirez *et al*., 2003) [12], and protection against radioactivity. Chemical components of the fruit and seed are mainly anthocyanins (in pulp) and other phenolics (Pepato *et al*., 2001) [10]. This study aimed to assess the anti-diabetic activity *in vitro* of plant resources used in traditional medicine in Siddha. The polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) was used to compare their antidiabetic and antioxidant properties *in vitro*.

Materials and methods

Extraction of polyphenol

Naval kottai chooranam (*Eugenia jambolan*) preparation, 100 g of the dried seed was crushed using blender to a paste-like state for 1 min. The homogenised sample was firstly freeze dried in order to reduce moisture content of the sample for a more efficient extraction process. The powder was then soaked in n-Hexane to defat for 24 h. It was then soaked in methanol for 72 h to obtain methanol crude extract, which was concentrated using a rotatory evaporator at 40 °C. The sticky residues were partitioned with chloroform to give chloroform soluble fractions. This was evaporated under reduced pressure and dried using an oven to obtain a polyphenol rich fraction by ethyl acetate (Dasgupta *et al.*, 2014).

Phytochemical Screening

The decoction of *Naval kottai chooranam* (*Eugenia jambolan*) were subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (Harborne 1973).

Thin Layer Chromatography

Thin layer chromatography of polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) was performed using standard procedures (Harborne 1973). The aqueous methanol extract was placed carefully in precoated aluminum silica gel 60 F, Merck F 254 using a microcapillary tube. The spots were allowed to dry for few minutes and the TLC plate was placed in the solvent mixture, Toluene, acetone and Formic acid (6:6:1) or solvents of ethyl acetate-glacial acetic acid-formic acid-water (100:11:11:26 v/v/v/v). After drying, the TLC plates were observed under UV at 240nm and 360 nm in UV TLC viewer.

α-Amylase Inhibition Activity

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 inhibitory activity was based on the starch iodine method that was originally developed by (Hamdan and Fatimai 2010) and later employed by others for determination of amylase activity in polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*). In alpha amylase inhibition method 1ml substrate- potato starch (1% w/v), 1 ml of methanol extract and ethyl acetate of different concentration such as 25, 50, 75 and 100 µg/ml, 1ml of alpha amylase enzyme (1% w/v) and 2ml of acetate buffer (0.1 M, 7.2 pH) was added. NOTE- Potato starch solution, alpha amylase solution and drug solution was prepared in acetate buffer (820.3 mg Sodium acetate and 18.7mg sodium chloride in 100ml distilled water).

$$\text{Inhibition of alpha- Amylase (\%)} = \frac{\text{Abs sample} - \text{Abs control}}{\text{Abs sample}} \times 100$$

B-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity was assessed by the standard method (Dong *et al*., 2012). Briefly, a volume of 25, 50, 75 and 100 µg/ml of sample solution and 50 µl of 0.1 M phosphate buffer (pH 6.8) containing α-glucosidase solution (0.2 U/ml) was incubated in test tube at 37 ºC for 20 min. After pre-incubation, 50 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside (PNPG) solution in 0.1 M phosphate buffer (pH 6.8) was added to each well and incubated at 37 ºC for another 20 min. Then the reaction was stopped by adding 160 µl of 0.2 M $NaCO_3$ into each well, and absorbance readings (A) were recorded at 405 nm by micro-plate reader and compared to a control which had 60 µl of buffer solution in place of the polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*). For blank incubation (to allow for absorbance produced by the extract), enzyme solution was replaced by buffer solution and absorbance recorded. The α-glucosidase inhibitory activity was expressed as inhibition % and was calculated as follows:

$$\text{Inhibition Percentage-} \frac{A_0 - A_1}{A_0} \text{X100}$$

Where, ACO is absorbance of the control and at is absorbance of the sample the concentration of inhibitors required for inhibiting 50% of the α-glucosidase activity under the assay conditions was defined as the EC_{50} value.

Inhibition of Albumin Denaturation Activity

The anti-inflammatory activity of polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) was deliberate by inhibition of albumin denaturation was studied. The reaction mixture consists of test extracts and 1% aqueous solution of bovine albumin fraction, pH of the reaction mixture was adapted using few drops of 1 N HCl. The differenrt concentration of alkaloid rich fraction were incubated at 37 °C for 20 min and then heated to 51 °C for 20 min, successively chilled the test sample was measured at 660 nm. The experiment was repeated in triplicate (Yang *et al*., 2010). The percentage inhibition of protein denaturation was calculated as follows:

Percentage inhibition= (Abs Control−Abs Sample) 100/Abs control.

Lox Inhibition

LOX is a relevant point in inflammatory processes. The LOX inhibitory activity was determined using a spectrophotometric assay (Perera *et al*., 2018) [11]. The method is based on increase of absorbance at 234 nm

because of the formation of 13-hydroperoxyocta-decadienoic acid in the lipoxygenation reaction. The mixture containing LOX solution (2200 U/mL) and borate buffer 0.2 M (pH 9.0) or extract in borate buffer was incubated for 15 min; then, linoleic acid was added to the mixture, and the absorbance was read at 234 nm for 2 min. Based on the % inhibition, the EC_{50} values were calculated for polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*). Diclofenac sodium was used as the standard.

Statistical Analysis

All data are expressed as the mean ± standard deviation (n = 3) and analyzed using Microsoft Excel. Statistical analysis was performed using Student's *t*-test, and the values were considered significant when $p < 0.05$.

Result and Discussion

Phytochemicals Properties

In this study, phytochemical screening of *Naval kottai chooranam* (*Eugenia jambolan*) was done to assess the availability of bioactive secondary metabolities. The presence of phytochemicals such as, flavonoids, alkaloids, tannins, steroids, phenols, saponins and terpenoids were detected.

Table 1: Phytochemical Screening of *Naval kottai chooranam* (*Eugenia jambolan*)

Sl. no.	Phytochemical Constituents	Observation	*Naval kottai chooranam* (*Eugenia jambolan*)
1	Alkaloids-Dragendorff's Test-Mayers test	Orange / red precipitate Yellow or	+
		white precipitate	+
2.	Flavonoids -Alkalai Reagent -Lead acetate test	Intense yellow colour	+
		Precipitate formed	+
3.	Glycosides Keller-Killiani test	Reddish brown colour ring formed	-
4.	Tannin -$FeCl_3$ test	Blue black coloration	-
5.	Saponins -Frothing test	Foam	+
6.	Terpenoids -*Salkowski test*	Dark reddish brown color in interface	-
7.	Polyphenols -Ferrozine test	Raddish blue	+
8.	Anthocyanin test Ammonia	Ammonia layer yellow in color	+

+ indicate positive result;-- Indicate negative result

TLC Profile

TLC analysis of polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) tested (Fig-1). In addition to the components with antidiabetic activity several compounds on the reference chromatogram were visible in UV light at 240 nm many of these compounds did not coincide with the antidiabetic a components. This could be qualified to evaporation of the active mechanisms, photooxidation or inadequate quantity of the active component (Masoko *et al*., 2005).

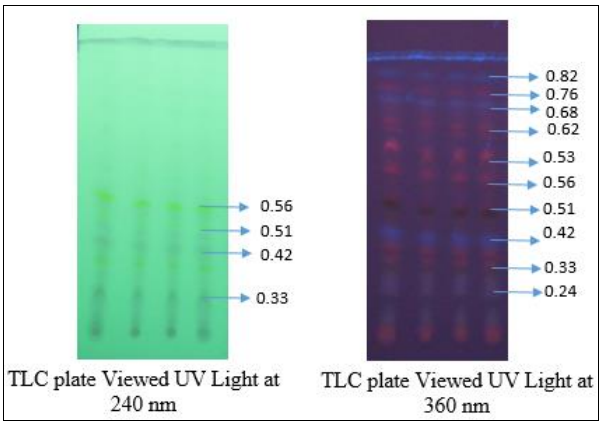

Fig 1: TLC analysis of polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*)

Inhibition of A-Amylase

Carbohydrate hydrolyzing enzymes, α-amylase digest dietary starch and degrade the oligosaccharides to glucose, resulting postprandial glucose surge. Therefore, inhibition of α-amylase activities is one of the primary approaches to manage hyperglycemic conditions of T2D patients. Acarbose is the most commonly prescribed digestive enzyme inhibitory drug. The inhibition of α-amylase, activity (EC_{50} values 64.23 µg/ml) by the tested plant extracts is described in Table-2. Polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) exhibited the highest inhibition percentage of α-alpha amylase 75.64% which was significantly efficacious compared to the drug, acarbose EC_{50} value (68.31µg/ml). The polyphenols are known to interact with the enzyme through non-specific binding, leading to inhibition of enzyme activity. The polyphenols tend to more effective on α-glucosidase inhibition with increase with molecular weight and degree of polymerization (Nistor Baldea *et al*., 2010).

Table 2: Inhibition activity of α-amylase by polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*)

Different concentration of extract	Inhibition percentage of protein denaturation	Diclofenac sodium (+ve control)
25 µl/ml	22.31±2.34	19.64±1.89
50 µl/ml	38.64±1.47	35.64±2.34
75 µl/ml	54.31±0.89	51.32±1.47
100 µl/ml	75.64±2.36	72.31±1.56
EC_{50} Value	64.23	68.31

Results are expressed as percentage inhibited amylase with respect to control. Each value represents the mean+SD of five experiments

Inhibition of A-Glucosidase

The results of *in-vitro* α-glucosidase inhibitory study are showed in Table-3. The polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) showed a concentration-dependent inhibition of enzyme. The highest concentration of 100 µg/ml tested showed a maximum inhibition of nearly 68.32% Polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) seems to be less potent in α-glucosidase inhibitory potential compared to Acarbose. It may be that α-glucosidase is more sensitive towards Acarbose with the concentration required for 50% inhibition (EC_{50}) found to be 76.32 mg/ml. Several lines of studies have indicated that the decline in pancreatic beta-cell

mass, through either an increase in apoptosis or a decrease in proliferation, is believed to be one of the major contributory factors in the development of type 2 diabetes (Baggio and Drucker, 2006)

Table 3: Inhibition activity of α-glucosidase by polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*)

Different concentration of extract	Inhibition percentage of protein denaturation	Diclofenac sodium (+ve control)
25 µl/ml	20.31±1.78	17.34±2.89
50 µl/ml	34.64±1.59	32.31±1.78
75 µl/ml	48.32±2.36	46.32±0.36
100 µl/ml	68.32±1.45	65.32±2.14
EC_{50} Value	72.31	76.32

Results are expressed as percentage inhibited amylase with respect to control. Each value represents the mean+SD of five experiments

Protein Denaturation Inhibition

Examination of polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) of momentous activity on inhibition of protein denaturation and its effect was compared with the standard drug Diclofenac sodium. The production of auto antigen in certain arthritic disease may be due to denaturation of protein. From the results of present study it can be stated that alkaloid extract is proficient of controlling the production of auto antigen and inhibits denaturation of protein in rheumatic disease. The maximum percentage inhibition of protein denaturation was observed as 67.32% at 100µg/ml which was close to the percentage of inhibition of diclofenac sodium 63.56%) (Table-4).

Table 4: Inhibition activity of protein denaturation by polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*)

Different concentration of extract	Inhibition percentage of protein denaturation	Diclofenac sodium (+ve control)
25 µl/ml	19.32±0.38	17.32±1.89
50 µl/ml	33.32±0.48	32.64±0.89
75 µl/ml	46.32±0.23	45.32±1.23
100 µl/ml	67.32±2.14	63.56±2.87
EC_{50} Value	75.32	82.34

Results are expressed as percentage inhibited inhibition of protein denaturation with respect to control. Each value represents the mean+SD of five experiments

Inhibition Activity of Lipoxygenase

The inhibition of LOX using linoleic acid as substrate was determined for the anti-inflammatory activity in the polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*). The extract at 100µl/ml concentration exhibited more inhibition than the other concentration. The inhibition percentage was lesser 62.31% at 100µl/ml (Table-5). The standard diclofenac sodium was showed 66.32% inhibition at 20 µg/mL. The polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*) was showed higher inhibition activity than positive control. Lipoxygenase catalyzes the addition of molecular oxygen to fatty acids containing a *cis*, *cis*-1, 4-pentadiene system. This reaction originates unsaturated fatty acid hydroperoxides. These products are further converted into others that play a key role in inflammatory processes.

Table 5: Inhibition activity of Lipoxygenase by polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*)

Polyphenol extract Concentration	Inhibition percentage of LOX	Diclofenac sodium (+ve control)
25 µl/ml	15.32±0.29	16.32±2.51
50 µl/ml	27.32±2.78	30.21±1.44
75 µl/ml	46.32±1.89	53.32±2.43
100 µl/ml	62.31±0.23	66.32±1.60
EC50 Value	83.32±0.23	78.32±1.23

Results are expressed as percentage inhibited Lipoxygenase with respect to control. Each value represents the mean+SD of five experiments

Conclusion

The present study provides the first pharmacological insight into the antidiabetic and anti-inflammatory potential of the polyphenol rich extract of *Naval kottai chooranam* (*Eugenia jambolan*). These traditional medicinal plant extracts also reduced significantly α-amylase and α-glucosidase activities compared to the most common drug, acarbose, indicating that the polyphenols present in the extracts have potential to reduce postprandial hyperglycemia by delaying the carbohydrate digestion. The antidiabetic ability to inhibit α-amylase, α-glucosidase enzymes, needed to be further explored using *in vivo* experimental models to validate the findings in the present study.

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