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ANTI-OXIDANT PROPERTIES OF A POPULAR TRADITIONAL POLYHERBAL ANTI DIABETIC FORMULATION - THETRAN KOTTAI KUDINEER

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ABSTRACT

Since ages humans have been dependent on plants as one of their primary source of food for survival. Plants are not only responsible for providing food but also they have many unexplored medicinal properties, humans have been able to harness only a very less proportion of its potentials to cure many deadly disease. The current trend of usage of synthetic drugs shifted to natural drugs as the former class of drugs have major side effects which are mostly life threatening, in order to nullify these side effects caused by the drugs to an extent scientists have shifted their focus on natural origin drugs as they are having very less side effects compared to synthetic origin drugs. Out of all the life threatening diseases which humans are facing by humans is diabetics, diabetics is an chronic disorder which is caused by impaired functioning of liver which fails either to produce insulin or reduce its sensitivity towards insulin as a result of which the blood glucose levels will rise than normal range. In traditional system of medicine there are many formulations which have been prescribed by traditional system of medicine practitioners, each and every formulation have its own mode and mechanism of action towards combating diabetes, one such formulation is thetran kottai kudineer which will render its action of anti-diabetics by anti oxidant activity but scientifically underexplored. Thus the present research is focused on the compilation of the standardization parameters carried out as per standards and also its potential anti oxidant activity by DPPH method.

KEY WORDS: Thetran kottai kudineer, antioxidant activity, DPPH method.

INTRODUCTION

Nature has bestowed human beings with an abundant of resources to survive in all the climatic conditions, out of all the resources available to human the widely used, exploited and essential one is Plants. Healing with medicinal plants is as old as mankind itself. The connection between man and his search for drugs in nature dates from the far past, of which there is ample evidence from various sources: written documents, preserved monuments, and even original plant medicines [1]. Diabetes is a group of metabolic disorders characterized by abnormal metabolism, which results most notably in hyperglycemia, due to defects in insulin secretion, insulin action or both. Diabetes comes from the Greek word for "siphon", and implies that a lot of urine is made. The second term, "mellitus" comes from the Latin word, "mel" which means

"honey", and was used because the urine was sweet. Diabetes mellitus is a global health crisis, which has been persistently affecting the humanity, irrespective of the socioeconomic profile and geographic location of the population. According to an estimate, one person is detected with diabetes every 5 seconds somewhere in the world, while someone dies of it every 10 seconds. Diabetes mellitus has attained a pandemic form [2]. Hence, it is very important to control diabetes and its complications to alleviate the human suffering.

Before the discovery of insulin in 1922, the only treatment options for diabetes were those based on traditional practices. Ethno botanical knowledge played a particularly important role in historical diabetes therapies, with over 1200 species of medicinal plants recognized throughout the world for their ability to treat diabetic

Indications [3]. Over the centuries, Thetran Kottai traditional decoction is using by the traditional practitioners to treat diabetes. This recipe contains a mixture of 4 plant ingredients: *Strychnos potatorum* (Family: Loganiaceae), *Cassia auriculata* (Family: Leguminoceae), *Terminalia chebula* (Family: Combretaceae) and *Limonia acidissima* (Family: Rutaceae) [4].

MATERIALS AND METHODS

Plant Material Collection & identification:

Fresh plant parts from plants of *Strychnos potatorum*, *Terminalia chebula*, *Limonia acidissima* and *Cassia auriculata* were collected in chilled container from local market in Puducherry. The various plant parts, i.e. leaves; seeds were thoroughly washed in cold water to remove the earthy material. The plant was identified with the help of *The Flora of Presidency of Madras* as reference book and authenticated by taxonomist, French Institute of Puducherry, India.

Preparation of Kudineer:

For the preparation of kudineer, 4.28 g of each ingredient was introduced into a clay pot and 1920 mL of tap water was added. Then it was boiled till the volume reduced to 240 mL using a low flame and filtered. The decoction was freeze dried and stored at 4 °C until use.

Qualitative evaluation of Phytochemicals

The morphological and microscopical examinations have been performed using reference book. The ash value, extractive value and preliminary phytochemical screenings were done using standard protocol to assess its quality and efficacy [5-6].

Investigation of antioxidant activity

DPPH radical scavenging assay

The antioxidant activity was determined by measuring the remaining concentration of DPPH' as described by Navarro and co-workers with some modifications [7]. In this assay, known concentrations of (0 - 50 µg/mL) decoction and butylated hydroxyl toluene

(BHT) were prepared in different test tubes by adding MeOH up to 1.5 mL. Three milliliters of methanolic solution of DPPH (2 mg/100 mL in MeOH) were added to these tubes and shaken vigorously. The tubes were allowed to stand at room temperature for 5 min. and the absorbance was measured at 517 nm. Control was prepared as above by adding MeOH instead of test solution. BHT served as positive control. This experiment was done in triplicates. The percentage of radical scavenging activity was calculated using the following equation [8].

$$\text{Percentage of Radical Scavenging Activity} = \frac{[(A_0 - A_s) / A_0] \times 100}{}$$

Where A_0 is the absorbance of the control and A_s is the absorbance of the sample at 517 nm. IC_{50} values denote the concentration of sample required to scavenge 50% DPPH' free radicals [9].

Statistical analysis of antioxidant activity

The following Data are given as Means + S.E.M. Statistical comparisons were made using one way ANOVA followed by Duncans Multiple Range Test. A P value < 0.05 was considered as significant. Decoction demonstrated a dose - dependent ($r^2 = 0.98$) DPPH' radical scavenging activity and comparable ($P > 0.05$) to the synthetic antioxidant, BHT [10]

RESULTS AND DISCUSSION

Total polyphenolic content and flavonoid content

The mean total polyphenolic content of the decoction was 80.45 ± 0.60 mg gallic acid equivalents/g extract respectively. The mean flavonoid content present in the decoction was 58.60 ± 0.70 mg quercetin acid equivalents/g extract respectively.

Physicochemical parameters

Physicochemical parameters like foreign matter, percentage of moisture content, total ash, acid insoluble ash, water soluble ash, ethanol soluble extractive and water soluble extractive were determined and depicted in Table.

Table 1. Phytochemical Screening of Extract of Thetran Kottai Kudineer

Constituents	Presence or Absence of Phytochemicals
Tannins	+
Steroid glycosides	+
Flavonoids	+
Alkaloids	+
Saponins	+
Anthraquinone Glycosides	-
Terpenoids	-
Protein	-
Carbohydrates	+
Volatile oils	-
Fixed oils	-

+indicates presence, - indicates absence

Table 2. Physico-chemical properties of Thetran Kottai kudineer

Parameters	Values obtained % w/w on dry weight basis
Foreign matter	Nil
Moisture content	7.47
Total ash	2.23
Water soluble ash	6.12
Acid soluble ash	0.08
Alcohol soluble extractive ash	5.65
Water soluble extractive ash	10.22

Table 3. Anti-oxidant activity of Thetran kottai kudineer

Sample	IC ₅₀ values (µg/mL)
Thetran kottai kudineer	12.5 ±0.06
Butylated Hydroxy Toluene (BHT)	11.3 ±0.08

Values are expressed as mean ± S.E.M., n =3

IC₅₀ value of the decoction was comparable ($P > 0.05$) to that of BHT

DISCUSSION

Reactive oxidants produced in biological systems, either by normal metabolic pathways or as a consequence of exposure to external agents have been associated with many different disease conditions such as cancer Alzheimer's disease and diabetes. In the present study, an attempt has been made to evaluate the antioxidant activity of the decoction by use of *in vitro* methods. The overall results of this investigation demonstrate that the decoction can exert significant antioxidant activity as evident from their ability to (a) scavenge free radicals such as DPPH and (b) presence of high content of poly phenols and flavonoids. Phytochemical screening of Thetran kottai kudineer revealed the presence of alkaloids, saponins, tannins, flavonoids and other phenolic compounds. The quantitative determination of phytochemicals also revealed that comparatively high amount of polyphenols and flavonoids are present in the decoction. Polyphenols are considered to be the major antioxidant compounds in plants, although they are not the only ones. The antioxidant activity of phenolic acids and flavonoids are reported to be mainly due to their redox properties which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Therefore, phenolic acids and flavonoids contained in this traditional decoction may play a major role in the observed antioxidant activities. Recently, much attention has also been focused on the role of oxidative stress in diabetes, and it has been suggested that oxidative stress may mediate the key and common events in the pathogenesis of different diabetic complications. Human

diabetics and experimental diabetic animal models exhibit high oxidative stress due to persistent and chronic hyperglycemia that may result in depletion of the antioxidant defence system and thus lead to an enhanced *de novo* free radical generation. Therefore, ability of the kudineer to inhibit generation of free radicals may also assist this plant to mediate its antidiabetic effects.

CONCLUSION

The present research work involved in the preliminary phytochemical evaluation and *in vitro* anti-oxidants activity of a popular, traditional, polyherbal antidiabetic Siddha formulation-Thetran Kottai Kudineer. The data obtained from the present findings clearly demonstrates that the Thetran Kottai Kudineer has excellent anti-oxidant properties which may be due to the presence of phytochemicals present in the formulation such as polyphenols and flavonoids. And the current research also paves a pathway to conclude the antidiabetic potential of Thetran Kottai Kudineer may act through free radical scavenging properties.

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