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Evaluation of Anti-Oxidant Activity of Siddha Herbo-Metallic Analgesic Drug "Ulogothama Chendhuram"

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ABSTRACT

BACKGROUND: Free radicals or highly reactive oxygen species are capable of inducing oxidative damage to human body. Antioxidants are the compounds which terminate the attack of reactive species and reduce the risk of diseases[1]. "Ulogothama chendhuram" (UC) used in treatment of degenerative diseases and have almost similar effects. OBJECTIVE: The study was conducted to determine the antioxidant properties of "Ulogothama chendhuram". RESULTS: The antioxidant activity of "Ulogothama chendhuram" was evaluated by measuring free radical scavenging activity by DPPH method. The antioxidant compounds like phenols and flavonoids were also evaluated in "Ulogothama chendhuram". The methanolic extract of Ulogothama chendhuram exhibited significantly higher antioxidant activity. The antioxidant compounds phenols and flavonoids were also found in "Ulogothama chendhuram". CONCLUSION: It can be concluded from the study that Ulogothama chendhuram an analgesic drug in degenerative diseases, which also have antioxidant property to prevent further degeneration.

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INTRODUCTION

Siddha Medicine is one of the ancient traditional medical systems that originated in southern part of India in Tamil Language. Siddhars made medicines by using herbs, metals, minerals and animal products. UC is one of the herbo-metallic preparations. It cures vatha diseases as indicated in siddha text pranarakshamirtha sindhu.

Vatha diseases also called arthritis. Arthritis occurs commonly in small joints of the hand, the vertebral column and the knees. The prevalence of arthritis especially osteoarthritis is on an alarming rise with a study saying we have over 180 million patients in India. Some below the age of 25.

Explain Dr. Shah said that "gum disease can also trigger arthritis". Mumbai–based orthopedic specialist Dr. CJ Thakkar observed a 20:1 ratio in females versus male patients." Women in the age bracket of 20-40 are seen to suffer from rheumatoid arthritis that affects multiple joints, while older women suffer from knee osteoarthritis due to the wear and tear of the knee joint. Add Dr. Kaushal malhan knee and hip replacement surgeon says "the increasing incidence of obesity has probably also contributed to the increasing incidence of osteoarthritis. "Genes play a huge role in early onset of arthritis". This may be the reason for a higher incidence of arthritis in woman at a younger age.

Experts maintain that arthritis can be tackled effectively with a judicious, better food habits, **higher antioxidant** content through green leafy vegetables, vitamin D and calcium foods[2].

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, cirrhosis, atherosclerosis, arthritis etc[3-4]. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders[5].

Antioxidant can protect against oxidative damage by decreasing the number of free radicals which cause chronic diseases and aging process[6].

On this aspect, "**UlogothamaChenduram"** is a herbo-metallic analgesic drug, and also containing higher anti-oxidant activity along with phenols and flavonoids.

Source of raw drugs:

The metal and mineral raw drugs were procured from a well reputed indigenous drug shop. The herbs were collected in Govt. Siddha Medical College, Chennai and Ariyalur (Dist.) Rayampuram. The drugs were authenticated by the pharmacognosist and chemist, Siddha Central research Institute, Chennai.

Ulogothama chendhuram (internal):

Ingredients:

- Vellai Pasanam (White Arsenic)
- Manosilai (Red Orpiment)
- Thaalagam (Arsenic Tri Sulphide)
- Rasa Karpooram (Mercurial Sub chloride)
- Lingam (Red Sulphide of Mercury)
- Gaantham *(Magnetic Oxide of Iron)*
- Gandhagam (Sulphur)
- Kuppaimeni Ilai charu*(Acalypha Indica)*
- Vetrilai Charu (Piper Betel*)*
- Paruthi Ilai Charu *(Gossipium Herbaceum*)
- Vellerukkam Ilai Charue (*calotropis gigantea*)
- Thulasi Ilai Charu (*Ocimum Sanctum*)
- Velliparuthi Ilai Charu (*Pergularia Daemia*)
- Poduthalai Ilai Charu (*Phyla Nodiflora)*

PREPARATION:

- The above said metals and minerals are first purified well as per classical textbook.[7]
- They are taken in equal proportion and grind it well with above said leaf juices for one day each. The whole content is made into a single poultrie; let it to dry in sun shade.
- This dried poultrie is covered with betel paste.
- Place this content in the mud pot covered with lid and sealed well with mud pasted cloth in seven layers.
- Then let it dry.
- Burn it in mild flame (Deepakkini) for one day.

- Chendhurum get sublimated in the upper lid.[8]

Preparation of the ulogothama chendhuram extract:

Preparation of the Ulogothama chendhuram extracts was assessed by following method as described by Janarthanam *et al.*, 2013. 0.5 gram of Ulogothama chendhuram dried powder of product materials were extracted with 20 mL aqueous for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotavator at 40°C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100 %. The solution was stored at 18°C until use.

Antioxidant activity in ulogothama chendhuram extracts

Qualitative analysis of antioxidant activity of ulogothama chendhuram extract

The antioxidant activity of Ulogothama chendhuram extract was determined by following method as described by George *et al.*,(1996); Selvaraj e*t al.*, (2013). 50µl of Ulogothama chendhuram extracts were taken in the microtiter plate. 100µl of 0.1% methanolic DPPH was added to the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of free radical scavenging activity of ulogothama chendhuram extract

The antioxidant activities were determined using DPPH (Sigma-Aldrich) as a free radical. 100µl of Ulogothama chendhuram extract were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (Lee *et al.*, 2005). Subsequently, at every 5 min interval, the absorption maxima of the solutions were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of 0.16% Butylated Hydroxy

Toluene (BHT). The experiment was carried out in triplicates. Free radical scavenging activity was calculated by the following formula:

$$\% \text{ DPPH radical -scavenging} = \frac{\text{(Absorbance of control - Absorbance of test Sample)}}{\text{(Absorbance of control)}} \times 100$$

Determination of total phenolic contents in ulogothama chendhuram

Total phenolic content in the aqueous Ulogothama chendhuram extract of was determined by the Folin Ciocalteau colorimetric method (Slinkard and Singleton, 1984). For the analysis, 0.5 ml aliquot of sample was added to 0.5 ml of Folin-Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of sodium carbonate (2%) was added, and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of total flavonoid content in ulogothama chendhuram

Total flavonoids content in the aqueous Ulogothama chendhuram extracts was determined by the aluminum chloride colorimetric method (Mervat *et al*., 2009). 0.5ml of Ulogothama chendhuram extracts at a concentration of 1mg/ ml were taken and the volume was made up to 3ml. Then 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent (QE) /g of sample.

RESULTS

Qualitative antioxidant activity of ulogothama chendhuram extract

S. No	**Extractions Control**	**Ulogothama chendhuram**
	BHT (standard)	++
	Control (DPPH)	-
S1	Aqueous (UC)	+

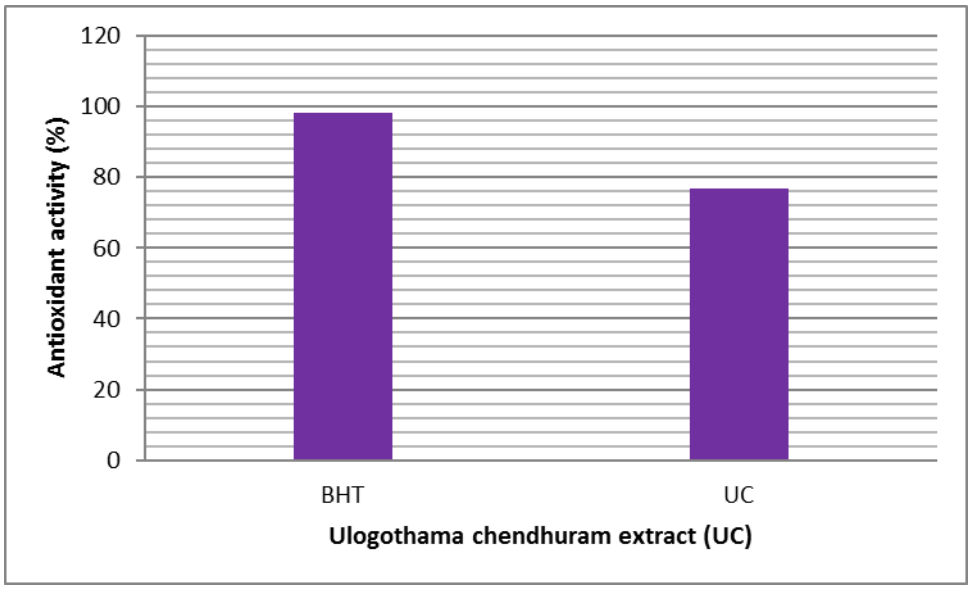
++ = strong positive; + = positive; - = negative

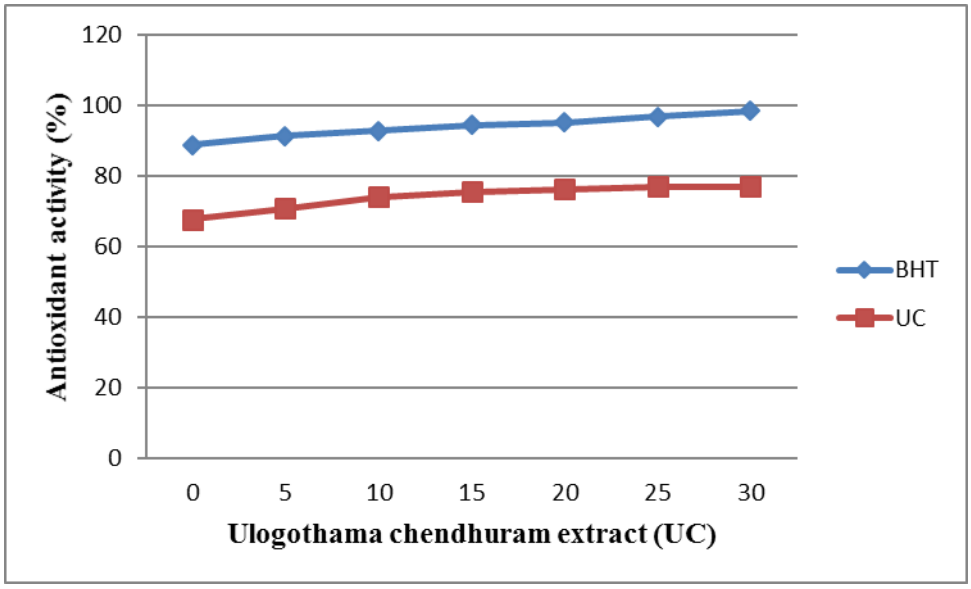
Quantification of phenol flavonoid of ulogothama chendhuram extract:

Quantification Phytochemical	**Ulogothama chendhuram**
Total phenolic content (mg GAE/g)	0.64
Total flavonoid content (mg GAE /g)	0.535

Quantitative analysis of free radical scavenging activity of ulogothama chendhuram extract:

Min	0	5	10	15	20	25	30
BHT (OD)	0.14	0.11	0.09	0.07	0.06	0.04	0.02
%	88.9	91.3	92.9	94.4	95.2	96.8	98.4
UC	0.41	0.37	0.33	0.31	0.30	0.29	0.29
%	67.7	70.8	74.0	75.5	76.3	77.0	77.0





DISCUSSION

Flavonoids are phytoconstituents which consist of large group of polyphenolic compounds having a benzo – y – prone structure and are abundantly present in plants. Flavonoids are proved for its high antioxidant activity *in-vitro* & *in-vivo*[8]. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals or by chelating metal ions[10]. Lipid peroxidation is a common consequence of oxidative stress. Flavonoid protects lipids against oxidative damage[9].

Most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. The capacity of flavonoids to act as antioxidant *in-vitro* has been the subject of several studies in the past years, and important structure-activity relationship of the antioxidant activity have been established[12].

Recent studies have been shown that polyphenolic constituents derived from plants are more effective antioxidants *in vitro* than Vit E or C[11]. The amount of flavonoids, phenolic compounds of UC has been showed in Table. Radical scavenging activity of UC has been shown in Fig. This shows that the antioxidant activity of UC is due to its presence of flavonoids, phenolic compounds in it.

CONCLUSION

Traditionally UC is used in the management of arthritis and other inflammatory conditions.

The present study has been conducted to evaluate the UC exhibited significantly higher antioxidant activity. The antioxidant compounds phenols and flavonoids were also found in "UC". So UC can help prevent further degeneration and management of arthritis.

This study highlights the role of degenerative and inflammatory cells, regulated free radical productions in the development of the pathology of arthritis and its treatment with UC. Antioxidant, anti-inflammatory, and analgesic activity of the UC extract may be attributed to the presence of flavonoids and phenols and it can be the treatment and prevention of arthritis.

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