



INVESTIGATIONS ON ANTI-OXIDANT, ANTI-ARTHRITIC AND ANTI-PLATELET STUDIES IN *COUROUPITA GUIANENSIS* AUBL LEAVES BY *IN-VITRO* METHODS

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ABSTRACT

The aim of the study was to investigate the anti-oxidant, anti-arthritis and anti-platelet activities in methanolic extract of *Couroupita guianensis* Aubl leaves (MECG) by *in-vitro*. Previous phytochemical analysis has indicated the presence of steroids, flavonoids and saponins. The MECGL showed astonishing anti-oxidant activity by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method, anti-arthritis activity by protein denaturation method and notable anti-platelet activities in all various concentrations i.e. 10, 50,100,200,400,800,1000µg/ml. The IC₅₀ value of MECG in DPPH radical was found to be 39.21 and ascorbic acid used as standard was found to be 41.43. The maximum protein denaturation was found to be 87.41% at a dose of 500mcg/ml and that of transmittance value for anti-platelet activity was found to be 84.41. The MECG showed good dose dependent activity in all *in-vitro* methods. The findings of the present study suggested that *Couroupita guianensis* could be a potential natural source of antioxidants anti-arthritis and anti platelet activities.

Keywords: Methanolic extract of *Couroupita guianensis* Aubl leaves (MECGL), anti-oxidant, anti-arthritis and anti-platelet studies, *in-vitro*.

INTRODUCTION

Free radicals play an important role in most major health problems such as Cancer, Cardiovascular Diseases, Rheumatoid Arthritis, Cataract, Alzheimer's disease and other Degenerative diseases associated with aging. Antioxidants are beneficial components that neutralize free radicals before they can attack cells and hence prevent damage to cell proteins, lipids and carbohydrates. Interest in the role of antioxidants in human health has prompted research in the fields of food science and medicinal herbs to assess the role of herbs as antioxidants^[1]. Rheumatoid Arthritis is an auto-immune disease and is closely related to synthesis of free radicals like, in the prostaglandin metabolism and synthesis of leukotrienes. One of the reasons of rheumatoid arthritis development is widely recognized the relation of free radical reactions in tissue injuries. Platelets play a central role in the process of blood clotting and are regarded as key regulators of both

haemostasis and pathogenesis of cardiovascular diseases. It is therefore important to prevent platelet dysfunctions that could lead to cardiovascular events^[2].

Couroupita guianensis is a large deciduous evergreen tree growing to a height of 20 meters. Leaves are alternate, oblong up to 20 cm long, entire to slightly serrate and hairy on the veins beneath. Inflorescence is racemose, arising from the trunk and other large branches. Flowers are reddish with a yellow tinge on the outside, fragrant, with stamens borne on an overarching androphore. Fruit is a large, reddish-brown globose, 15 to 24 cm, with a woody capsule, and each containing 200 to 300 seeds. This plant is used for treating mange and other skin conditions. The pulp of the fruit of the cannon ball tree is rubbed on the infected skin of mange dog. It is claimed that when the dog licks its skin, this medicine will also work internally. The flowers are used to cure cold, intestinal gas formation and stomach ache. The leaf has been found to show anti oxidant activity, anthelmintic activity, immuno modulator and anti-nociceptive activity^[3-6]. So far no systematic study has been reported for antioxidant by DPPH method, anti arthritic activity by protein denaturation and anti platelet activity by measuring transmittance values in UV spectrophotometer.

MATERIALS AND METHODS

Plant materials:

The leaves of *Couroupita guianensis* were collected in Venkittampalayam village near Thiruvannamalai district, Tamil Nadu in the month of Dec 2011. The specimen was identified and authenticated by Prof. Dr. P.Jayaraman, Director, Plant Anatomy Research Centre (PARC), Chennai. The specimen was deposited to herbarium of Anurag Pharmacy College. After authentication, fresh leaves collected in bulk from plants, washed, shade dried and then milled to a coarse powder by a mechanical grinder,

Preparation of extract:

The powders of dried leaves were packed in to soxhlet column and extract with ethanol. The extract was filter through a Whatman filter paper no.1 and concentrated under reduced pressure (yield of extract was 9.40% with respect to dry material).

***In-vitro* antioxidant activity by DPPH method**

Determination of DPPH· scavenging activity DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity was carried out according to the methods described earlier

(22–24). Briefly, 1 mL of DPPH· (500 mM in ethanol) was added to a mixture of test samples (10–100mL) and 0.8 mL of Tris-HCl buffer (pH=7.4). After vigorous shaking, the mixture was allowed to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm with a UV-VIS spectrophotometer. Ascorbic acid was used as positive control. Radical scavenging potential was expressed as IC50 value, which represents the sample concentration at which 50 % of the DPPH· radicals were scavenged^[7]. The results were tabulated in Table 1 & Figure 1.

In vitro anti arthritic activity by inhibition of protein denaturation method

1. **Test solution** (0.5ml) consists of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of test solution (250µg/ml).
2. **Test control** solution (0.5ml) consists 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 0.05ml of test solution (250mcg/ml).
4. **Standard solution** (0.5ml) consists of 0.45ml of bovine serum albumin (5% w/v aqueous solution) and 0.05ml of Diclofenac sodium (250µg/ml).

All of the above solutions were adjusted to pH, 6.3 using a small amount of 1N HCl. The samples were incubated at 37°C for 20minutes and heated at 57°C for 3 minutes. After cooling, add 2.5ml of phosphate buffer to the above solutions. The absorbance of the above solutions was measured using UV-Visible spectrophotometer at 416nm. The percentage inhibition of protein denaturation was calculated using the formula.

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

The control represents 100% protein denaturation. The results were compared with Diclofenac sodium (200µg/ml) treated samples^[8,9]. The results were tabulated in Table 2 & Figure 2.

***In-vitro* Anti-platelet activity**

The platelet rich plasma 0.13×10^7 for each assay was re-suspended in pH 7.4 Tyrode buffer. The platelet aggregation was recorded as transmittance values of spectrophotometer measurement. To determine the *in-vitro* inhibition of platelet aggregation different concentrations of MECG like 50, 100, 200, 400 $\mu\text{g/ml}$ in DMSO were used. The platelet aggregation was induced with ADP at a concentration of $5 \mu\text{M}$ which is used as control^[10]. The Aspirin $100 \mu\text{g/ml}$ is used as a standard. The transmittance is recorded at interval of 1 min for every 5 min. The results were tabulated in Table 3 & Figure 3.

RESULTS AND DISCUSSION

DPPH radical scavenging assay is one of the most widely used methods for screening of antioxidant property of plant products. DPPH is stable nitrogen centered free radical and can easily abstract an electron or hydrogen radical from the suitable reducing agents to become a stable diamagnetic molecule. The unpaired electron of DPPH thus gets paired off forming the corresponding non-radical hydrazine. The radical scavenging property of the sample was determined by measuring the decrease in absorbance of DPPH. The dose dependent inhibition of DPPH radical indicates that MECG can reduce DPPH radical in a stoichiometric manner and this radical scavenging effect may be due to its hydrogen donating property. The MECG showed good antioxidant activity in DPPH scavenging method. Percentage inhibition are tabulated in 1 and was found to be quite equivalent to that of the standard, Ascorbic acid. IC₅₀ (Inhibitory concentration at which there is 50% reduction in free radical reaction) values was found to be Reducing power of the extract was dose dependent which was indicated from the increase in absorbance with the increase in concentration of the extract (Figure 1).

TABLE 1: *IN-VITRO* ANTIOXIDANT ACTIVITY BY DPPH SCAVENGING METHOD

Concentration in mcg/ml	Standard (Ascorbic acid)	MECG
50	20.93 ± 0.17	18.96 ± 0.21
100	34.21 ± 0.24	26.71 ± 0.13
150	52.62 ± 0.13	49.82 ± 0.24
300	74.81 ± 0.12	72.49 ± 0.12
500	91.27 ± 0.18	87.21 ± 0.21

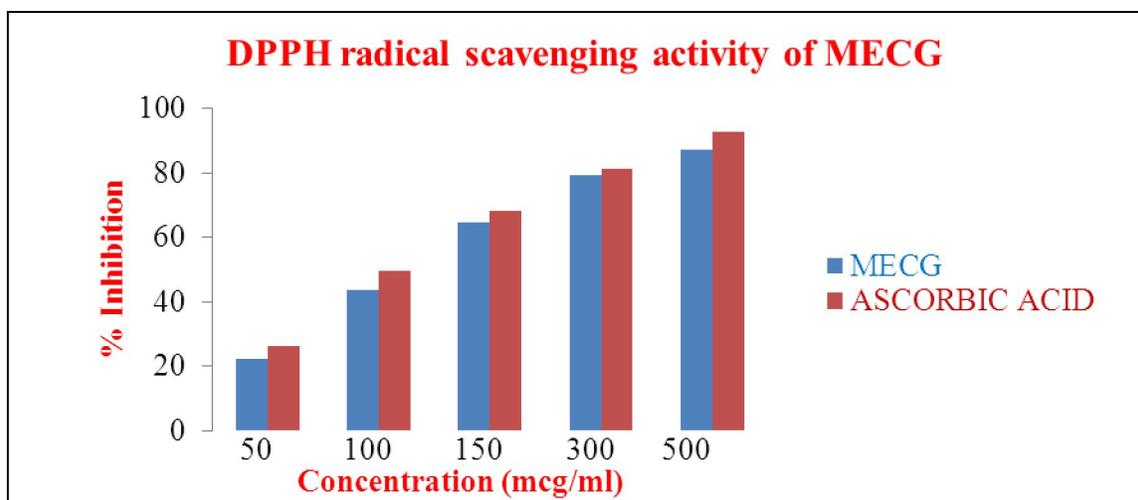


Figure 1

In-vitro antioxidant activity by DPPH scavenging method

Anti-arthritic effect of MECG was studied significantly by using *in-vitro* inhibition of protein denaturation model. The effect of MECG on inhibition of protein denaturation is shown in Table 1. MECG at different concentrations (50, 100, 150, 300 and 500µg/ml) provided significant protection against denaturation of proteins. Most of the investigators have reported that denaturation of protein is one of the cause of rheumatoid arthritis. Production of auto-antigens in certain rheumatic diseases may be due to *in vivo* denaturation of proteins. Mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding. The maximum protein denaturation inhibition of MECG was found to be at 87.41 % (Figure 2).

TABLE 2: IN VITRO ANTI-ARTHRITIC ACTIVITY BY PROTEIN DENATURATION METHOD

Concentration in mcg/ml	Standard (Diclofenac sodium)	MECG
50	26.29 ± 0.21	22.47 ± 0.21
100	49.62 ± 0.13	43.71 ± 0.32
150	68.21 ± 0.22	64.65 ± 0.14
300	81.29 ± 0.21	79.27 ± 0.12
500	92.87 ± 0.14	87.41 ± 0.25

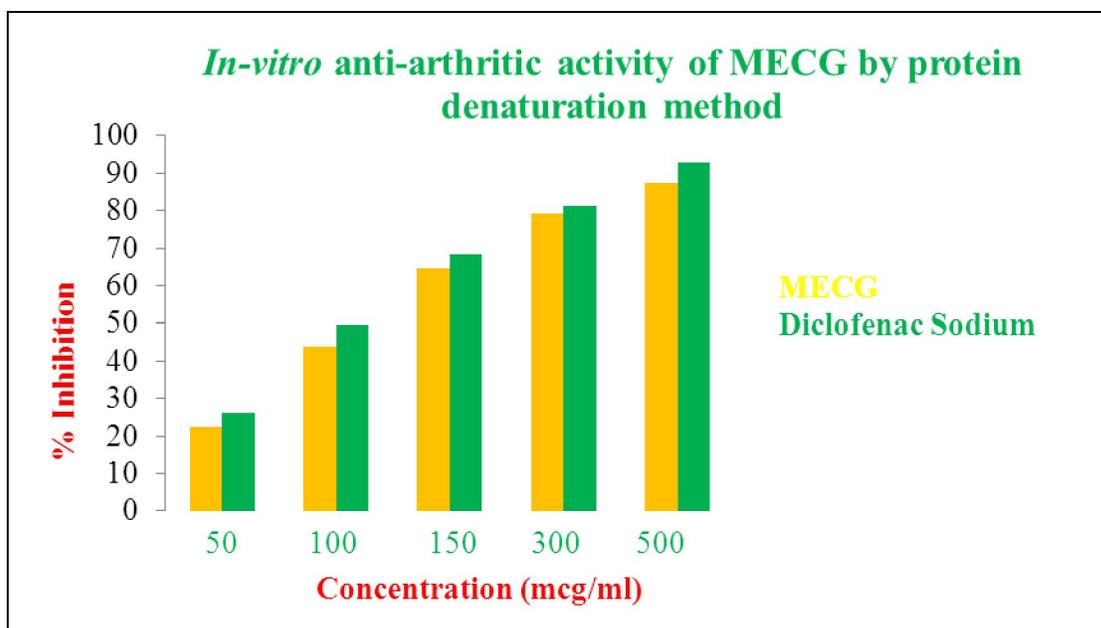


Figure 2

% Inhibition of MECG for *In vitro* anti-arthritis activity by Protein denaturation method

Platelets activation do not play key role in homeostasis, moreover their hypersensitivity is reported to be related with development and progression of atherosclerosis. The MECG showed dose dependent inhibition of ADP-induced human platelet aggregation and more than 20% inhibition were observed at the initial dose of 25 $\mu\text{g/ml}$. The MECG showed dose dependent inhibition of ADP-induced human platelet aggregation. The results are tabulated in Table 3 and % inhibition was shown in Figure 3.

TABLE 3: %TRANSMITTANCE VALUES FOR *COUROUPITA GUIANENSIS* LEAVES

Concentration in $\mu\text{g/ml}$	%Transmittance values in minutes						
	0	1	2	3	4	5	
ADP (5 $\mu\text{g/ml}$)	28.417	32.218	30.283	29.412	26.371	24.541	
ASPIRIN(100 $\mu\text{g/ml}$)	92.162	81.291	70.481	64.261	54.161	52.112	
Plant extract	25	48.141	39.132	32.121	29.142	27.241	24.182
	50	51.213	47.264	41.231	38.492	32.432	25.163
	100	62.421	55.121	47.451	39.211	37.291	27.138
	200	76.232	68.232	61.232	54.231	49.182	42.264
	400	84.413	79.212	74.323	68.432	64.173	61.351

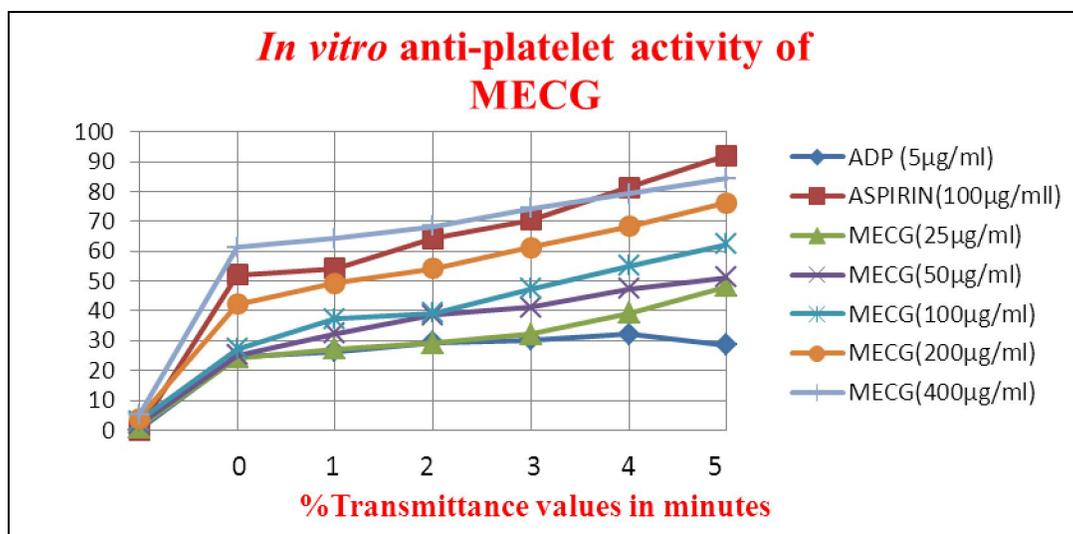


Figure 3
in vitro anti platelet activity of MECG

CONCLUSION

Phytochemical analysis reveals the presence of carbohydrates, alkaloids, phenolic compounds, tannins and saponins. So the secondary metabolites of *Couroupita guianensis* may be responsible for the anti-denaturation effects. The methanolic extracts of *Couroupita guianensis* demonstrated the anti-denaturation effect at lower concentration therefore the leaf extract may possess antioxidant, anti-arthritic and anti-platelet activity. Further *in-vivo* animal experiments and phytochemical investigation are required to prove the biological activity like anti-arthritic and responsible secondary metabolite respectively.

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