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"PHYTOCHEMICAL & PHARMACOLOGICAL INVESTIGATION OF Cassia siamea LAM. FLOWERS."

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ABSTRACT

Present investigation includes evaluation of free radical scavenging potential of *Cassia siamea* lam. (*C.siamea*) belonging to family Fabaceae, an important traditional medicinal herb in the Indian system of medicine. The dried powdered material of *C.siamea* flowers macerated to prepare total methanolic extract and solvent extraction technique prepared various fractions such as n-hexane fraction, ethyl acetate fraction and Chloroform fraction. Experimental design to screen extract and various fractions for its free radical scavenging potential by determining Hydroxyl radical scavenging activity, Superoxide radical scavenging activity, Phosphomolybdenum Assay (PM), Cupric ion reducing capacity assay (CUPRAC). Ascorbic acid was used as a standard drug. The results were revealed that C.siamea flowers have significant antioxidant potential when compared with standard. Our finding suggested that, the ethyl acetate fraction of *C.siamea* flowers having better efficacy and significant antioxidant activity. This work enlightens the antioxidant potential of this plant and also helps to support the traditional medicinal claim and believes of this plant in therapeutics.

Keywords: *Cassia siamea*, Fabaceae, Free radicals, antioxidant activity.

INTRODUCTION:

Nature has been a source of medicinal agents and a lot of amount of new drugs have been isolated from natural sources; among them many herbs are used as traditional medicine. Among that Cassia species (Caesalpinaceae) are well known medicinal plant commonly found in India and other tropical countries. Different medicinal properties have been attributed to this plant in the traditional system of Indian medicine. Various anthraquinones have been isolated from the seeds of Cassia species.

Cassia siamea is a shrub belonging to the Fabaceae family, native of Southeast Asia and better known in folklore medicine, feeding, agriculture and manufacture all over the world. *C. siamea* has recently been shown to have antimicrobial, antimalarial, antidiabetic, anticancer, hypertensive, diuretic, antioxidant, laxative, anti-inflammatory, analgesic, antipyretic, anxiolytic, antidepressant, and sedative activities. Chromone (anhydrobarakol), Chromone alkaloids (barakol, cassiarin A-B), anthraquinones (chrysophanol, emodin), bianthraquinones (cassiamin A-B), flavonoids and phenolics compounds are the main constituents which are reported in this plant. Barakol was identified as the major constituents of *C. siamea* of leaves and flowers of the world.

MATERIALS & METHODS:

1. Collection of Plant material

The flowers of *Cassia siamea* Lam. were collected from village of Bangarwadi near the campus of Samarth Rural Educational Institute, Belhe and Pune 412-410.

2. Authentication of Plant

India in the month of September 2019 the Plant would be authenticated from Sangamner Nagarpalika Arts, D.J. Malpani commerce, B.N. Sarda Science College, Sangamner, Ahmednagar from Botany Department.

3. Preparation of powder

The plant materials (flowers petals) were dried under shade at our Pharmacognosy Laboratory for about four weeks and then made into powdered form, using mortar and pestle and then sieved.

4. Extraction of plant material

The dried powdered material of flowers (500 g) was extracted with methanol & covered with aluminium foil used to prevent evaporation of the solvent. Place at room temperature by cold maceration techniques with continuously shaking and stirring. After maceration sample get filter by Whatman No. 41 filter paper. Three macerates prepared and combined to get total methanolic extracts. The extract was evaporated to dryness under reduced pressure below 40◦C.The some portion of total Methanolic extract was further fractionated by solvent-solvent with n-hexane, chloroform, ethyl acetate in the order of their increasing polarity to obtain respective fractions.

5. Qualitative & Quantitative phytochemical tests

The qualitative & quantitative phytochemical test of total methanolic extracts such as n-hexane fraction, ethyl acetate fraction and chloroform fraction, & methanolic Extract of *C. siamea* flowers was carried out using standard procedure.

6. Antioxidant Activity:

Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity of the extractives was determined by the method of Halliwell et.al. The assay was performed by adding 0.33mL of phosphate buffer (50mM , pH 7.9), 1.0mL of the extract of different concentration (20, 40,60,80 & 100µg/mL) dissolved in distilled water, 0.1mL EDTA, 0.01mL of $FeCl_3$, 0.36mL of deoxyribose. The reaction mixture kept in water bath at 37^{o}c for 1 hour. Reaction is started when addition of 0.1mL of ascorbic acid & then 0.1mL H_2O_2. After incubation add 1.0mL of 0.5% cold TBA (Thiobarbituric acid) & then 1.0mL of 25% HCL (Hydrochloric acid). The mixture again heat at 100^{0}c for 15 min. Cooled down on water bath. To develop the pink chromogen & the absorbance was measured at 532nm.

Determination of Superoxide Radical Scavenging Activity:

The superoxide anion scavenging activity was measured as described by Srinivasan et al. The superoxide anion radicals were generated in 3.0 mL of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 mL NADH (0.936mM) solution, 1.0 mL extract of different concentration (20, 40,60,80 & 100µg/mL), and 0.5 mL Tris – HCl buffer (16mM, PH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25^{o}C for 5 min and the absorbance was measured at 560 nm against a blank sample, ascorbic acid.

Cupric ion reducing capacity assay (CUPRAC)

Cupric ion reducing capacity was measured in accordance to the method of Apak R., et.al. 1 ml 10 mM cupric chloride, 1 ml 7.5 mM neocuproine and 1 ml 1 M ammonium acetate buffer of pH 7 solutions were added to test tubes containing 2 ml of distilled water. Various fractions of *C. siamea* flowers in different concentration ranging from 20µl to 100µl were added to each test tube separately. These mixtures were incubated for half an hour at room temperature and measured against blank at 450 nm. Ascorbic acid was used as positive reference standard. All methods were repeated in triplicate in order to get mean value.

Phosphomolybdenum Assay (PM)

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto (1999). Methanolic extract of *C. siamea* flowers in different concentration ranging from 20µl to 100µl were added to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. These tubes were kept incubated at 95^0c for 90 min. After incubation, these tubes were normalized to room temperature for 20-30 min and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as positive reference standard.

Statistical analysis:

The percentage inhibition was measured by comparing the absorbance values of control and test compounds, whereas ascorbic acid was taken as standard. The % of inhibition was calculated by using equation:

% Inhibition=

[(Absorbance control – Absorbance sample)/Absorbance control] x 100.

RESULT & DISCUSSION:

In the present study, the powdered material of flowers was extracted and extractive values of total Methanolic extract and its fractions such as n-hexane fraction, Chloroform fraction, ethyl acetate fraction where found to be 3.12 %w/w, 1.4 %w/w, 1.92 %w/w, 2.5 %w/w respectively.

Preliminary phytochemical analysis showed total Methanolic extracts of *C. siamea* flowers revealed the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds. The n-hexane fraction shows positive result for the Phytosterols. Ethyl acetate fraction showed positive test for shinoda & Lead acetate test indicated presence of rich in flavonoids. Chloroform fraction showed positive for $FeCl_3$ test indicated abundance of phenolic compounds.

Phenolic and flavonoids are the major constituents in plant extracts responsible for its antioxidant activity. So, the results obtain in this study showed a significant level of phenolic, flavonoids& tannins compound in Methanolic extract, n-hexane, Chloroform, Ethyl acetate fractions of the flowers of *Cassia siamea*. The total phenolic content was observed to be maximum in methanolic & ethyl acetate fraction of *C.siamea* flowers. Also the flavonoids content was found to be more in methanolic & ethyl acetate fraction and significantly lower in n-hexane & chloroform fraction. Similarly Tannins content was found to be maximum in methanolic & ethyl acetate fraction & minimum in remaining solvent.

The results of in vitro assays showed the total methanolic extract of *C.siamea* flowers (Group I) and its fractions includes n-hexane fraction (Group II), Chloroform fraction (Group III) and Ethyl acetate fraction (Group IV) significant inhibition and free radical scavenging potential when compared with standard ascorbic acid (Group V). It indicated that extract and its fractions seem to be significant anti-lipid peroxidation potential as compared to ascorbic acid as a standard. However comparative evaluation of total methanolic extracts and fractions, it was prominently noted that ethyl acetate fraction of *C.siamea* flowers indicated better significant free radical scavenging activity and anti-lipid peroxidation efficacy as compared to other fraction and extract as shown in table.

Table: Antioxidant activity of *C.siamea* flowers

Group	Treatment	% inhibition			
		Hydroxyl radical scavenging activity	**Superoxide radical scavenging activity**	**Cupric ion reducing capacity assay (CUPRAC)**	**Phosphomolybdenum Assay (PM)**
I	Total Methanol extract	60.90 ± 0.015**	52.09 ± 2.971*	49.64 ± 0.721*	73.67 ± 0.96**
II	n- hexane Fraction	19.46 ± 0.017*	11.08 ± 0.502ns	10.85 ± 0.23*	12.16 ± 0.38*
III	Chloroform Fraction	21.06 ± 0.041*	13.75 ± 0.036*	12.78 ± 0.47*	14.65 ± 1.240*
IV	Ethyl Acetate Fraction	63.84 ± 1.551**	58.81 ± 1.839**	52.24 ± 0.025**	77.00 ± 0.11**
V	Standard	75.71 ± 2.040	73.67 ± 0.96	80.07 ± 0.64	84.73 ± 0.25

Values are expressed as mean ± SEM, n=6. When Group (I, II, III, IV) compared with Group (V); *P<0.05, **P<0.01, ***P<0.001 symbols represent statistical significance; ns - not significant.

However comparative evaluation of total methanolic extracts and fractions, it was prominently noted that ethyl acetate fraction of *C. siamea* flowers indicated better significant efficacy as compared to other fraction and extract because it may related to presence of some chemical constituents. Ethyl acetate fraction showed positive test for shinoda & Lead acetate test indicated presence of rich in flavonoids. The Antioxidant activities of the flower extracts were found to be in the order of Ethyl acetate >Methanol>chloroform> n-hexane as shown in figure.

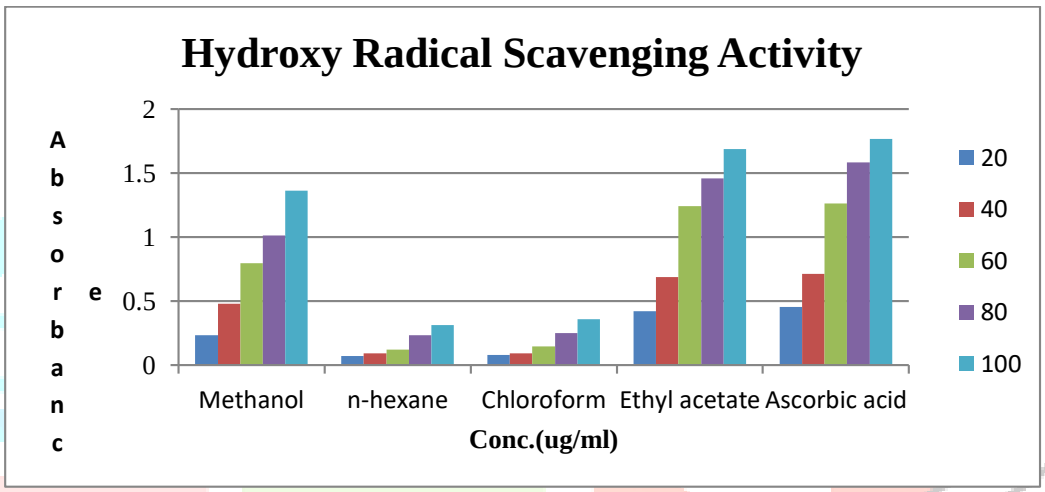

Figure: Hydroxyl radical scavenging activity

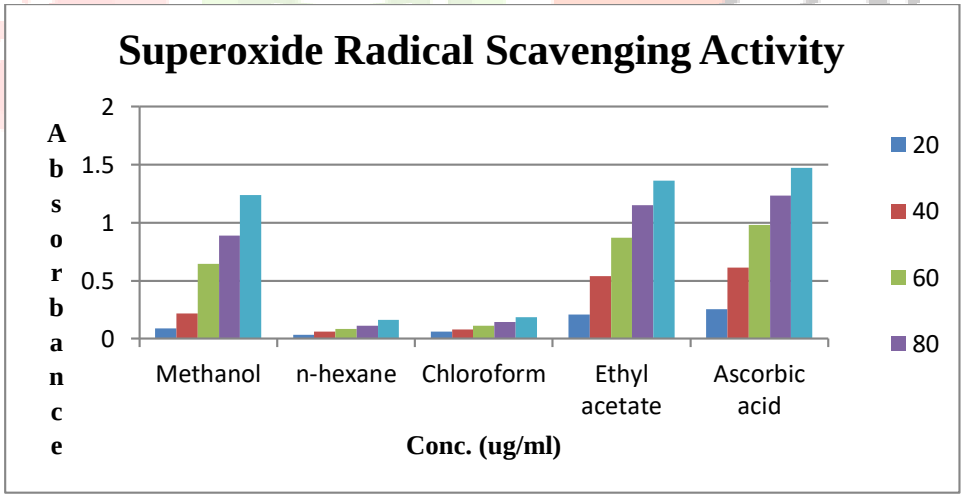

Figure: Superoxide radical scavenging activity

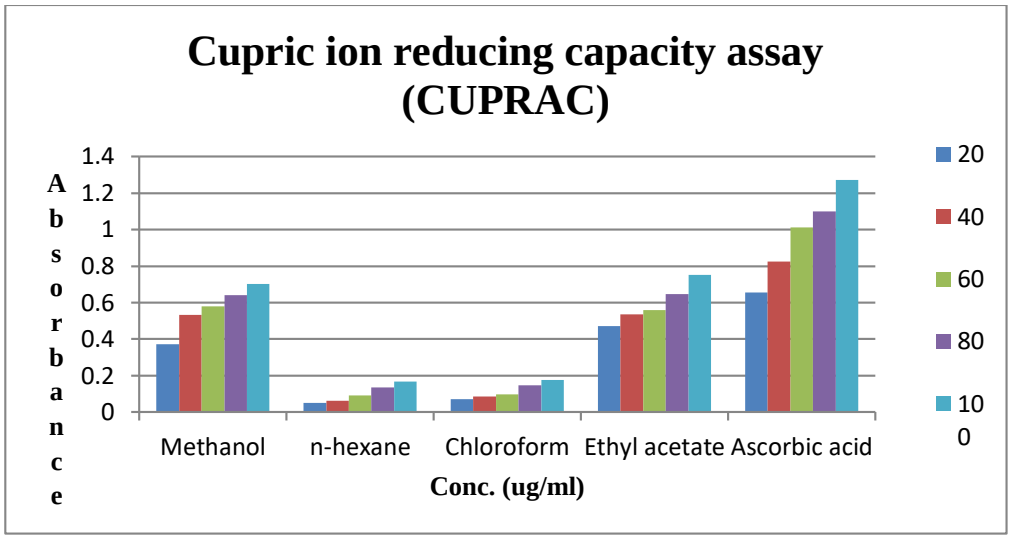

Figure: Cupric ion reducing capacity assay (CUPRAC)

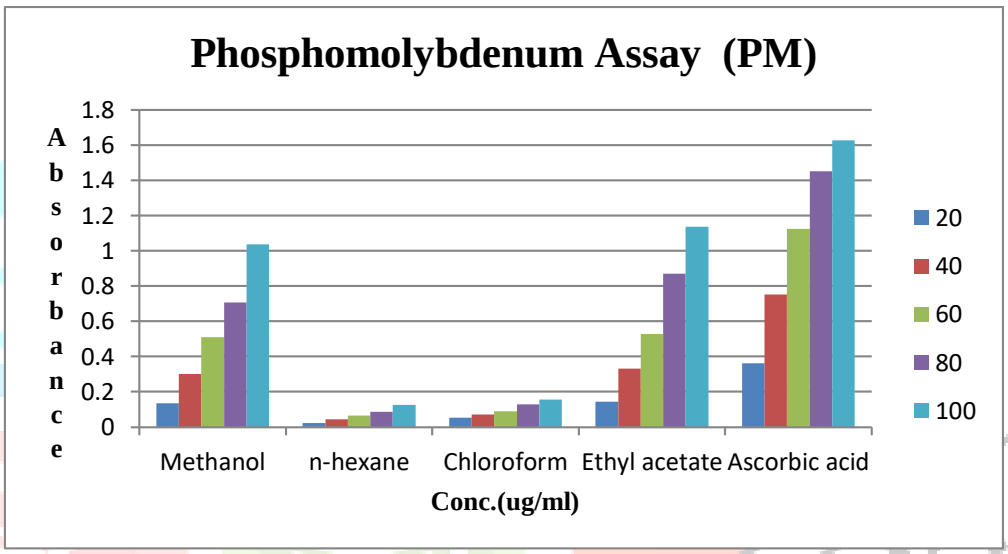

Figure: Phosphomolybdenum assay

From investigation, it can be concluded that *C. siamea* lam. contains some significant phytochemicals & with the help of different antioxidant capacity assays, the ethyl acetate extract of *Cassia siamea* Flowers has shown a significant total antioxidant capacity.

CONCLUSION:

In conclusion, it could be stated that, among the various solvent extracts of *Cassia siamea* L. flowers analysed for preliminary phytochemical screening. The methanolic extract followed by the successive methanolic fractions were found to contain more phytoconstituents than other solvent extracts which might be due to the polarity and extracting ability of methanol. From the investigation of qualitative& qualitative phytochemical analysis, flowers of *Cassia siamea* L. was found to be a good source of beneficial phytoconstituents which may have a prominent Antioxidant activity.

From our findings, it can be concluded that *C. siamea* lam. contains some significant phytochemicals& with the help of different antioxidant capacity assays, the ethyl acetate extract of *Cassia siamea* Flowers has shown a significant total antioxidant capacity. Thus, this study gives support the traditional believes of this medicinal plant.

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