IN-VITRO ANTI-OXIDANT AND ANTI-MICROBIAL STUDY ON *CASSIA AURICULATA* LINN.

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ABSTRACT

The extensive list of disorders and pathogenesis in which radicals and oxidants have been implicated is still growing. From the past decades the herbal products are widely used in traditional and ayurvedhic formulations. The herbal products probably have not shown any sign of toxic to human beings also with lesser or no side effects. The study was intended to evaluate the in-vitro antioxidant and antimicrobial activity of methanolic extract of *Cassia auriculata* Linn. (MECA) in leaves, fruits and flowers were determined and each extracts was measured using three different methods of nitric acid radical, DPPH (1,1-diphenyl-2-picryl-hydrazyl) and hydrogen peroxide scavenging assay while the ascorbic acid and rutin were used as standard and positive control for the analysis. Further they have studied about the flavonol content and antimicrobial activity in different strains. The MECA had shown the significant activity in all the methods when compared to the standard antioxidants i.e., ascorbic acid, rutin. Eventhough, among the three methods and different extract, the fruits of MECA showed that maximum reducing power of inhibition in the investigation also fruit MECA found that 46.1% in nitric acid, 69.5% in DPPH, 79.0% in hydrogen peroxide method. The antioxidant activity was confirmed by the presence of the flavonoids presence hence the flavonol content also measured in the content and showed 18.55%. Methanol extract of fruits of *C. auriculata* showed the highest antioxidant activity while the methanol extract of leaves and flowers of *C. auriculata* possessed the lowest antioxidant activity. However, the finding showed that most of the tested extracts were showing strong antioxidant activity even higher than the ascorbic acid and rutin.

Keywords: *Cassia auriculata*, DPPH, nitric acid, hydrogen peroxide, antimicrobial activity.

INTRODUCTION

Reactive oxygen species (ROS) is a term that encompasses all highly reactive, oxygen-containing molecules, including free radicals and the hydroxyl radical. The super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical and various lipid peroxides. ROS is capable of reacting with membrane lipids, nucleic acid, proteins, enzymes and other small molecules, resulting in cellular damage. Cell membranes are made up of unsaturated lipids; while the unsaturated lipid molecule particularly susceptible to damaging free radical process and readily contributes to the uncontrolled chain reactions[1]. Reactive oxygen species and reactive nitrogen species are endogenous intermediates, constantly produced in the human body, while they are components of the signaling cascade involved in cellular oxido/redox status toward a more oxidative stress, results in

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pathological manifestations such as atherosclerosis, cancer, inflammatory condition, diabetes, alzheimer's disease and parkinson's disease like about 50 diseases. Eventhough various sources of the anti-oxidants are available in herbal remedies such allium sulphur compounds, anthocyanins, beta-carotene, catechins, copper, cryptoxanthins, flavonoids, indoles, isoflavonoids, lignin, lutein, lycopene, manganese, polyphenols, selenium, vitamin C & E, zinc and zoo chemicals etc., those the all components widely provided by various see foods, lean meat, milk, nuts, pumpkin, mango, cabbage, cauliflower, spinach, carrot, grapes, berries, garlic, onions, apples and wine etc., The plant genus *Cassia auriculata* Linn. having a wide range of pharmacological actions. The *C. auriculata* leaves constituted polysaccharides, flavonoids, anthracene derivatives and dimeric procyanidins[2]. The plant was reported with spectral elucidation of di-(2-ethyl) hexyl phthalate[2]. The plant flowers were reported having anti-oxidant The plant *C. auriculata* constitutes pyrrolozidine alkaloids, roots constitutes flavones glycoside, bark includes tannins and flowers β-sitosterol and seed fatty oils consisting of palmitic, oleic and linoleic acids. The plant is traditionally used to treat fever, leprosy, eye injuries, whoophing cough, chest disease, diabetes, conjuctivities, skin diseases and jaundice[2]. Thereby, the all specific pharmacological active plant was intended to evaluate a detailed study on anti-oxidant and antimicrobial activity.

1. *Materials and methods*

1.1. *Plant Collection, Authentication and Extraction*

The plant was collected from Mukombu, and surrounding area of Tiruchirappalli. The collected plant was authenticated by Botanical Survey of India, Coimbatore. Voucher No. BSI/SC/5/25/07-08/Tech. The parts such as leaves, flowers and fruits were separated and shade dried. The dried parts were chopped separately using methanol by cold maceration. The extracts were concentrated to dryness under reduced pressure and controlled temperature and it was stored in desiccator for further studies[3].

1.2. *Phytochemical Screening*

Phytochemical screening was evaluated the presence of alkaloids, aminoacids, carbohydrates, flavonoids, glycosides, proteins, saponins, steroids, tannins and terpenoids in MECA of leaves, fruits and flowers[4].

1.3. *Scavenging of Nitric acid radicals*

The reaction mixture 6ml containing sodium nitroprusside (10mM) 4ml, PBS 1ml and 1ml of extract in DMSO were incubated at 25ºC for 150 minutes. After incubation 0.5ml of the reaction mixture containing nitrate was removed and 1ml of sulphanilic acid was added, mixed well and allowed to stand for completion of diazotization, then 1 ml of napthyl ethylene diamine dihydrochloride was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance of the solution was measured at 540 nm using ELISA reader against corresponding blank solution and IC_{50} value calculated.

1.4. *Scavenging of Di-Phenyl Picryl Hydrazyl (DPPH) radical*

The assay was carried out in a 96 well in micro titrate plate and 200 µl of DPPH solution, 10µl of each test sample or the standard solution was added separately in wells of the microtitre plate. The final concentration of the test and standard solutions used are 1000 to 1.95 µg/ml. the plates were incubated at 37ºC for 20mins the absorbance of each solutions were measured at 490nm using ELISA reader

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against corresponding blanks and the remaining DPPH was calculated. IC_{50} is the concentration of the sample required to scavenge 50% of free radicals and IC_{50} was calculated[5].

1.5. Scavenging of hydrogen peroxide radicals

A solution of Hydrogen peroxide 20mM was prepared in phosphate buffer saline (pH 7.4). various concentrations of 1ml of the extracts or standard in methanol were added to 2ml of hydrogen peroxide solutions. The absorbance was measured at 230nm after 10 mins against a blank solution which contained extracts in PBS without hydrogenperoxide and IC_{50} value was calculated.

1.6. Calculation

The IC_{50} value was calculated by following formula.

$$IC_{50} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100$$

1.7. Anti-bacterial and anti-fungal activity

Anti-bacterial and anti-fungal activity was carried out by employing 24 hour cultures of *Bacillus aerogenus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Klebsiella pnemoniae, Fungal strains, Aspergillus flavuns, Candida albicans, Trychopthyton* activity of the MECA was tested separately by using agar well diffusion method. The medium was sterilized by autoclaving 120ºC (15 lb/in^2) above 30 ml of medium with the respective strain of bacteria and fungi was transferred aseptically into each sterilized petriplates. The plates were kept at room temperature for solidification each plate, a single well of 6 mm diameter was made using a sterile borer. The extracts were freshly reconstituted with suitable solvents.

RESULTS

Table .1
Extraction of different parts of the plant

S. No.	Part of the Plant	Weight of the Sample used (g)	Quantity of Methanol (ml)	Weight of crude extract obtained (g)
1.	Leaves	100	250	4.792
2.	Flower	100	250	3.945
3.	Fruit	100	250	3.823

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Table .2
Qualitative phytochemical screening of MECA

S. No	Tests	Leaves	Flowers	Fruits
1.	Alkaloids	-	-	-
2.	Amino acids	+	-	+
3.	Carbohydrates	+	-	+
4.	Flavonoids	+	+	+
5.	Glycosides	+	-	+
6.	Gums and mucilages	-	-	-
7.	Proteins	+	-	+
8.	Saponins	+	+	+
9.	Steroids	+	+	+
10.	Tannins	+	+	+
11.	Terpenoids	-	-	-

Table .3
Total Flavonol Estimation of different parts of MECA

S. No.	Plant extract	Total Flavonol content (%)
1.	Leaves	7.5
2.	Flower	14.59
3.	Fruit	18.55

Table .4
Anti-oxidant activity of different parts of MECA using Nitric acid radical inhibition, DPPH and Hydrogen peroxide method (IC_{50} Values)

S. No.	Plant parts extracts used	IC_{50}		
		Nitric acid method	DPPH method	Hydrogen peroxide method
1.	Leaves	10.4±1.75	25.2±2.51	72.0±2.53
2.	Flower	27.2±3.26	80.5±31.4	46.5±3.45
3.	Fruit	46.1±4.54	69.5±15.5	79.0±15.50
4.	Ascorbic acid	15.5±2.33	34.1±9.27	54.1±9.27

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5.	Rutin	28.8±6.85	23.6±1.79	43.6±1.79

Table .5

Determination of MIC by two fold serial dilution methods

S. NO.	Minimum inhibitory Concentration (µg/ml)			
	Bacterial strains	**Leaves**	**Flower**	**Fruit**
1.	*Bacillus aerogenus*	>1000	125	62.5
2.	*Bacillus coagulans*	500	62.5	62.5
3.	*Bacillus subtilis*	125	62.5	31.25
4.	*Staphylococcus aureus*	>1000	125	31.25
5.	*Escherichia coli*	125	15.5	15.5
6.	*Klebsiella pnemoniae*	62.5	15.5	15.5
	Fungal strains	**Leaves**	**Flower**	**Fruit**
1.	*Aspergillus flavuns*	>1000	1000	500
2.	*Candida albicans*	1000	250	125
3.	*Trychopthyton ligonium*	125	62.5	15.5

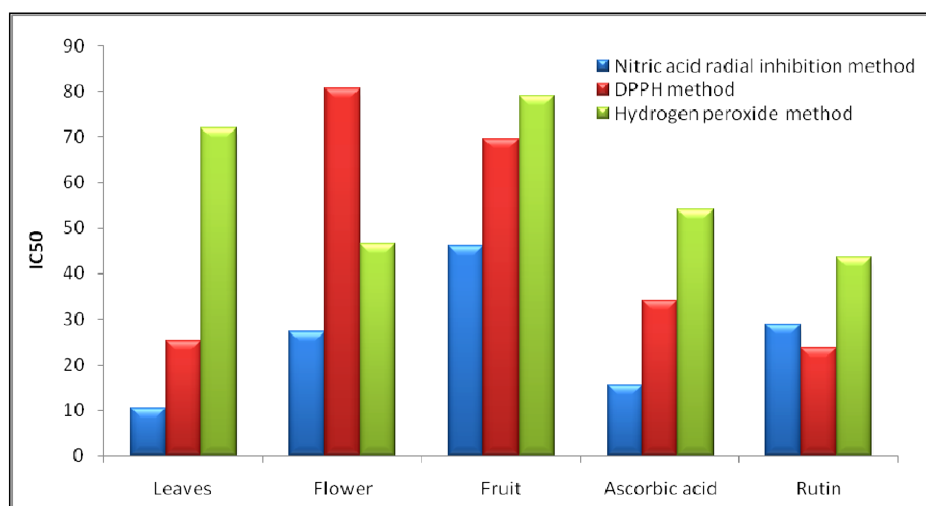

Fig.1

Comparative evaluation of anti-oxidant activity of MECA

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DISCUSSION

The preliminary phytochemical studies were showed that the presence and absence of the chemical compounds in the aerial parts of MECA separately in (Table.2.). Such that the flavonoids, saponins, steroids and tannins were present in all parts of the MECA. The flavonol content was found that 7.5% in leaves, 14.59% in flower and 18.55% in fruit by which the plant was showed that higher value in fruit of MECA (Table.3.). The antioxidant activity was found that the fruits of the *C. auriculata* showed maximum percentage of inhibition (Table.4.). In Nitric acid free radical scavenging method the plant showed that maximum activity on fruit of MECA showed (46.1) and rutin (28.8). In this method leaves and flower of MEC and ascorbic acid showed very less action to the antioxidant activity. In DPPH method showed that flower, fruit and ascorbic acid showed that maximum activity, particularly the flower of MECA showed (80.5) inhibiting concentration on best significant value than standard antioxidant as ascorbic acid. In final method, the hydrogen peroxide method the fruit of MECA showed (79.0) inhibiting concentration higher than the standards as ascorbic and rutin. Also, the leaves of MECA showed (72.0) inhibiting concentration. In this three methods the fruit of MECA showed significant inhibition in the oxidants, such that we can conclude that the flavonol content was stimulating the antioxidant property by (18.55%). Also, the previous literature showed that the anti-inflammatory and antioxidant activities are correlated, by which the plant genus showed anti-inflammatory activity. So, the plant has studied the antioxidant property also it shown the significant results in different three methods. The number of sugar residues at the C3 position seems to be very important for antioxidant activity. The smaller the number of sugar units at C3, the higher the antioxidant activity. Also, it is reported that the stability of aryloxyl radical affected the antioxidant activities of compounds and may give rise to pro-oxidant effects[6]. So, the leaves and fruit of MECA showed that presence of sugar as carbohydrates and glycosides. The IC_{50} values represent the concentrations of the test compound that gave half-maximal activity under the standard assay conditions. Further, the antimicrobial activity was conducted on different bacterial and fungal species mentioned in the (Table.5.) *Bacillus aerogenus, Staphylococcus aureus, Aspergillus flavuns, Candida albicans* showed that the maximum inhibiting concentration about 1000 µg/ml. In this antimicrobial activity the leaves of MECA only showed that maximum significant value than the fruits and flowers of MECA. From the present study leaves, flowers and fruits of MECA showed antioxidant and antimicrobial activity, it can be used for the further study.

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