

Study of Antioxidant Activity and Immune Stimulating Potency of the Ethnomedicinal Plant, *Cassia alata* (L.) Roxb.

Saheli Chatterjee¹, Sabyasachi Chatterjee², KK Dey³ and Sikha Dutta^{1*}

¹Department of Botany, The University of Burdwan, India

²Department of Biotechnology, The University of Burdwan, India

³Department of Surgery, Burdwan Medical College and Hospital, Burdwan, India

Abstract

Today there is renewed awareness of the value of natural resources, and this realization has led to carrying out tests for an increased utilization of wild plants as food and drug sources [1-4]. *Cassia alata* (L.) Roxb. (Leguminosae family) is a medicinal plant, whose English names are Ringworm bush and Candelabra bush. It is a native plant of South America and can be found widely in tropical region. Fresh or dried leaflet of *Cassia alata* has been used as folk medicines in many countries for treatment of constipation, stomach pain, and ringworm and skin disease [5]. In present study, an attempt has been made to investigate the antioxidant potency and the immune stimulating property of this ethnomedicinally important plant *Cassia alata*. Methanolic extract of the leaves of *C. alata* was assayed for determining the antioxidant compounds present in this plant. Estimation of total phenols, Vitamin-C, Vitamin-A, flavonoids, carotenoids and anthraquinones was done. DPPH radical scavenging activity of the methanolic extract of leaves was also tested against a synthetic antioxidant, Butylated hydroxytoluene (BHT). It is evident from the results that the plant *Cassia alata* possesses strong antioxidant activity, as it contains good quantity of antioxidant compounds like phenols, Vitamin-C, Vitamin-A, flavonoids, carotenoids and anthraquinone. In addition, it has very high DPPH radical scavenging activity in contrast to the synthetic antioxidant compound, BHT. The plant *Cassia alata* has strong immune-modulating or immune-stimulating potency, as evidenced by a steep rise in the total count of leucocytes with concomitant increasing in granulocyte: a granulocyte ratio as well as remarkable increase in the total number of peritoneal macrophages in the rabbits treated with the aqueous extract of leaves of *C. alata*. Thus, the plant *Cassia alata* may extensively be used in therapeutic medicines as a resource of natural antioxidants and immune stimulating agent.

Keywords: *Cassia alata*; Antioxidant activity, Immune stimulating property; Phenols; Flavonoids; Vitamin-A; Vitamin-C; Anthraquinones; DPPH radical scavenging activity

Introduction

Free radicals contribute to more than one hundred disorders in human beings, including atherosclerosis, arthritis, ischemia, central nervous system disorders, gastritis, cancer and AIDS [6]. Free radicals due to environmental pollutants, radiations, Chemicals, toxins, deep fried and spicy foods, as well as physical stress, cause depletion of immune system, change in gene expression and induce abnormal proteins. Due to depletion of immune system, natural antioxidants in different maladies consuming antioxidants as free radical scavengers may be necessary [7].

An increasing interest in the search for natural replacements of synthetic antioxidants has led to the antioxidant evaluation of a number of plant sources. The nutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health benefit presents distinct challenges in evaluating antioxidant activity. Thus, there is a renewing interest in phytomedicine during last decade, and nowadays many medicinal plant species are being screened for its pharmacological potential.

India is the major exporter of raw MAP'S (Medicinal and Aromatic Plants) and processed plant based drugs. About 45,000 plant species with medicinal properties have been assigned to several thousand for the turnover of herbal medicines in India. However, very few plant species have been thoroughly investigated for their medicinal properties.

Recently, there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well-known and traditionally used natural antioxidants from tea, wine, fruits, vegetables, spices,

some natural antioxidant are already exploited commercially either as antioxidant additives or a nutritional supplements [8]. Also, many other plant species have been investigated the search for novel antioxidants [9], but generally there is still a demand to find more information concerning the antioxidant activity of plants might be due to their phenolic compounds [10]. The number of reports on isolations of natural antioxidants, mainly of plant origin, has increased immensely during the last decade [11]. Phenolic compound are commonly produced in plants as a general line of their natural defence.

They have multiple applications in food, cosmetic and pharmaceutical industries [12]. The antioxidant capacity of phenolic compound is mainly due to their redox properties, which allow them to cut as reducing agents, hydrogen donor's singlet oxygen quenchers or metal chelators. In addition to their roles as antioxidants, these compounds exhibit a wide spectrum of medicinal properties, such as anti-allergic, anti-inflammatory, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effect [13].

Cassia alata (belonging to the family Leguminosae) (Figure 1) has been reported to have various phytochemical activities. Pharmacological investigations performed so far on *Cassia alata* have revealed that this

***Corresponding author:** Dr. Sikha Dutta, Department of Botany (CAS), The University of Burdwan, Burdwan-713104, West Bengal, India, Tel: +91-9434672206; E-mail: sikha_bu_bot@yahoo.com; bubotdrsd@gmail.com

Received June 11, 2013; Accepted July 23, 2013; Published July 26, 2013

Citation: Chatterjee S, Chatterjee S, Dey KK, Dutta S (2013) Study of Antioxidant Activity and Immune Stimulating Potency of the Ethnomedicinal Plant, *Cassia alata* (L.) Roxb.. Med Aromat Plants 2: 131. doi:10.4172/2167-0412.1000131

Copyright: © 2013 Chatterjee S, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

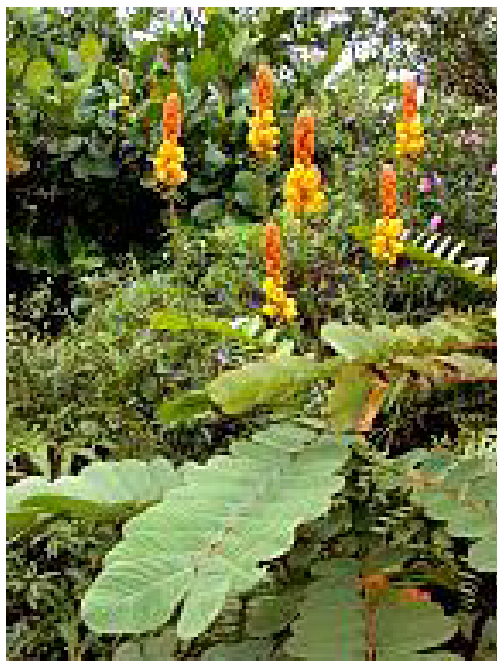


Figure 1: *Cassia alata*

herb has several biological activities, such as antimicrobial [14,15], purgative [16], anti-inflammatory [17,18], analgesic [17,19,20], hypoglycaemic [17], and antitumor activities [21].

Considering the growing demands of herbal medicines and those too natural antioxidants, the objective of the present investigation was to determine the antioxidant potential and immune stimulating property of the common ethno-medicinally important plant, *Cassia alata*.

Materials and Methods

Sample collection

The plant *Cassia alata* was collected from road side of Tarabag, the residential campus of Burdwan University, Burdwan, and West Bengal, India.

Determination of antioxidant property of *Cassia alata*

Leaf extracts of *C. alata* were used for determination of antioxidant properties of the plant. Estimation of different antioxidant compounds and assay of DPPH radical scavenging activity were studied. Methanolic extract of leaves was used for determination of antioxidant properties of this plant (Figure 2).

Preparation of alcoholic extract

Leaves dried at room temperature were taken and put in boiling methanol at a rate of 5-10 ml of methyl alcohol per g of tissue and allowed to boil for 5 to 10 minutes, in order to have extraction on a steam bath or hot water bath under a hood. It was then cooled in a pan of cold water, crushed thoroughly in a mortar and pestle for 5 to 10 minutes, and filtered through two layers of cheese cloth. The residue was again extracted with 2 to 3 ml of alcohol for 3 minutes in a hot water bath, cooled and passed through cheese-cloth. Both the extracts were mixed and the mixture was filtered through Whatman No. 1 filter paper. The alcohol from the extract was then evaporated till the volume was reduced by 80-90%.

Estimation of total phenols: Total phenol was estimated using Folin-Ciocalteu reagent [22]. For this purpose, alcoholic extract of plant tissue was used.

Reagent preparation: Folin Ciocalteu reagent was made by dissolving 100.0 mg of sodium tungstate and 25.0 gm of sodium molybdate in 700.0 ml of distilled water. 50.0 ml of 85% ortho-phosphoric acid and 100.0 ml of conc. HCl were added to it and boiled under reflux gently for about 10 hours and allowed to cool. 150.0 gm of lithium sulphate dissolved in 50.0 ml water and 4-5 drops of liquid bromine together were added to it gently. The mixture was boiled without condenser for about 15 mins to remove the excess bromine, cooled and diluted to 1000 ml with water and filtered. The reagent should be golden yellow in colour and stable for several months, and stored in amber coloured bottles. Just before use, the reagent was diluted with one volume of this stock solution with 2 volumes of water.

Estimation: For estimation of total phenol, 1 ml of alcoholic extract was taken in a graduated test tube and 1 ml of Folin Ciocalteu reagent was added to it, which was followed by the addition of 2 ml of 20% Na_2CO_3 solution. The tube was shaken and placed in a boiling water bath, for exactly 1 min., cooled under running tap water. The blue solution developed was diluted with distilled water up to 25 ml, and its absorbance was recorded at 650 nm in a spectrophotometer (Simadzu). Total phenol was calculated from a standard curve prepared from catechol. Blank set was made identically. Total phenol content was expressed in terms of ($\mu\text{g/ml}$) of extract.

Estimation of flavonoids: 5 ml of sample of the alcoholic extract of leaf was taken in a test tube containing 1.25 ml of distilled water [23]. Then 0.075 ml of 5% sodium nitrite solution was added in it and allowed to stand for 5 minutes. Subsequently, 0.15 ml of 10% Aluminium chloride (AlCl_3) was added to the mixture. After 6 minutes, 0.5 ml of 1 M sodium hydroxide was added, and the mixture was diluted with another 0.275 ml of distilled water. The absorbance was measured at 510 nm in a spectrophotometer (Simadzu). A standard curve was prepared



Figure 2: Inflorescence of *C. alata*

from analytical grade Catechin. And the total flavonoids content in the leaf tissue of *Cassia alata* was calculated, and was expressed in terms of ($\mu\text{g/ml}$) of extract.

Estimation of Vitamin-C: Ascorbic acid was estimated following the method described by Oser [24]. 100 mg of fresh leaf tissue was homogenized with 5 ml of 6% trichloro acetic acid (TCA). After centrifugation at 5000 rpm for 5 min., the supernatant was collected and a pinch of activated charcoal (Norit) was added to it and filtered. The volume of the filtrate was made up to 100 ml with addition of distilled water. 4ml of supernatant was taken in a test tube and 2 ml of 2% 2,4 dinitrophenyl hydrazine and one drop of 10% thiourea solution (in 70% alcohol) were added to it. Dinitrophenyl hydrazine solution was prepared in another conical flask by adding 2 gm of dinitrophenyl hydrazine into 100 ml of 9(N) H_2SO_4 (concentrated). The mixture was then boiled for 15 min. in a water bath. After cooling at room temp., 5 ml of 80% H_2SO_4 was added in the mixture at 0°C. After waiting for 30 min, the absorbance of the solution was taken at 530 nm in a spectrophotometer (Simadzu). A standard curve was prepared from analytical grade ascorbic acid, and the Vitamin C content in the leaf tissue of *Cassia alata* was calculated and was expressed in terms of ($\mu\text{g/ml}$) of extract.

Estimation of Vitamin-A: Vitamin A was estimated by method of Carr and Price [25]. 100 mg of fresh leaf tissue sample was weighed and ground well using mortar and pestle. To this, 1 to 2 ml chloroform was added and then centrifuged, supernatant was collected, and it was made up to 50 ml with distilled water. This served as test solution was taken in series of test tubes and made up to 3 ml with chloroform. Blank was also setup with 3 ml of chloroform, 0.2 ml of test solution was taken and made up to 3 ml with chloroform. To all the test tubes, 2 ml of antimony trichloride was added. The blue colour developed was read at 620 nm within 15 seconds.

Standard vitamin A solution: About 40 to 50 mg of a vitamin A concentrate, which comes in gelatin capsules, Control KO. PC-3 from Distillation Products, Inc., were diluted in chloroform to give a concentration of vitamin A (as the alcohol) from 2 to 5 micrograms per ml of solution. Standard curve was drawn. Vitamin-content in the leaf tissue of *Cassia alata* was calculated, and was expressed in terms of ($\mu\text{g/ml}$) of extract.

Estimation of total carotenoids: Extraction and estimation of total carotenoids was done following the method of Thimmaiah [26]. 1 gm of the fresh leaf tissue sample was homogenized with peroxide free ether, until it was free from pigments, filtered and the filtrates were pooled and partitioned with equal quantity of peroxide free ether. The ether phase which contains the carotenoids was evaporated and the residue was dissolved in 1 ml of ethanol, and absorbance was measured at 450 nm. The carotenoids present in the extract were calculated from the A_{450} values using the following formula:

$$C=D \times V \times F / 2500$$

Where, C=Total amount of carotenoids (mg)

D=Absorbance at 450 nm

V=Volume of the original extract in ml

f=Dilution factor and

2500=Average extinction coefficient of the pigments

Estimation of anthraquinone: Sample preparation: *Cassia alata* leaf powder (100 mg) was extracted with methanol (20 ml) under reflux



conditions for 1 hr. The extract was then filtered and concentrated. The sample was adjusted to 5 ml with methanol [27].

Conformity test for anthraquinone: Anthraquinone, which is the major component of *Cassia alata*, is identified by this procedure [28]. Borntrager's reaction was used to detect anthraquinone in the extract. HCl (2 N) was added to the sample, and the mixture was heated on a hot water bath for 15 min, then cooled and filtered. The filtrate was mixed well with chloroform layer and was separated with it. The mixture was shaken with 10% KOH solution, the aqueous layer become pink-red, which confirms the presence of anthraquinone in the sample (Figure 3).

Method of estimation of anthraquinone in the extract: Crude extract was taken and mixed with 30 ml water, weighed and refluxed for 15 min. The aqueous mixture allowed to cool, weighed and adjusted with H_2O to the original weight. The sample was centrifuged at 4000 rpm for 10 min. 10 ml of supernatant was taken with 20 ml of 10.5% $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and refluxed for 2 min where aglycones and glycosides are produced. These were extracted with 25 ml \times 3 ml of ether. The ether layer and the aqueous layer formed separately. The ether layer was washed separately with water (15 ml) for 2 times and adjusted to 100 ml with ether. 25 ml from the ether layer was taken and evaporated to dryness. The residue was dissolved with 10 ml of 0.5% Magnesium acetate in methanol and absorbance was taken at 515 nm, and calculated the percentage (%) of total anthraquinones and expressed in terms of ($\mu\text{g/ml}$). Standard curve was prepared from sodium anthraquinone-2-sulfonate (analytical grade, SIGMA-ALDRICH).

Assay of DPPH free radical-scavenging activity: The antioxidant activity was determined according to the DPPH radical scavenging assay. The effect of the methanolic extracts of leaf, flower and pods of *C. alata* on 1, 1-diphenyl-2-picrylhydrazyl (DPPH, Fluka Chemie) was estimated following the method, as described by Brand-Williams et al. [29]. Samples were diluted in methanol prior to the analysis (1 mg/ml). The DPPH solution was added to the diluted samples, thoroughly mixed, then left for 30 min for the reaction to occur. After that, the absorbance of the samples was measured at 515 nm using a UV-Vis

spectrophotometer (Simadzu). The absorbance of DPPH solution in methanol, without any antioxidant (control), was also measured. The percentage of DPPH radical scavenging activity was calculated by using the following equation:

$$\text{DPPH scavenging (\%)} = \frac{(\text{A}_{\text{control}} - \text{A}_{\text{sample}})}{\text{A}_{\text{control}}} \times 100$$

Where, a sample is the absorbance of the sample after the time necessary to reach the plateau (30 min), and a control is the absorbance of DPPH. Extract concentrations providing IC₅₀ inhibition values (defined as the concentration of the compounds that was able to inhibit 50% of the total DPPH radicals) were calculated from graph plotting using nonlinear regression, and expressed in microgram material equivalents per milliliter for sample extracts. Butylated hydroxytoluene (BHT) was used as a positive control. A lower value of IC₅₀ indicates a higher antioxidant activity and *vice versa*.

Study of immune stimulating activity of *C. alata*

For assay of immune modulating/stimulating activity of *C. alata*, aqueous extract of leaves of *C. alata* was prepared following standard protocol, and was injected to adult rabbit with the help of a hypodermic syringe at a dosage of 10 ml aqueous extract per kg of body weight. Control group of rabbit were maintained which received no of leaf extract of *C. alata*. Estimation of haemoglobin, total count, differential count, count of peritoneal macrophages was done at an interval of 7 days up to 21 days.

Estimation of haemoglobin

Haemoglobin content was estimated following acid haematin method, in which haemoglobin gets converted into acid haematin by the action of hydrochloric acid, and was measured using haemoglobinometer. Content of haemoglobin was expressed as g/100 ml blood.

Total count of Leucocytes (TC)

It was done by haemocytometer method. For calculating total leucocyte content, blood was diluted with WBC diluting fluid, which

removes the red cells by haemolysis, and also accentuates the nuclei of the white cells. Counting was done under a microscope, and by knowing the volume of the fluid examined and the dilution of the blood, the number of white cells per cu mm in undiluted whole blood was calculated.

Count of leucocytes (DC or granulocyte-agranulocyte ratio)

It was done following the Leishman stain method. Blood sample smear was prepared and observed under microscope. Approximately, 100 leucocytes were counted and from that observation, the ratio of granulocyte and agranulocyte was calculated.

Count of peritoneal macrophages

Count of peritoneal macrophages was done following the method of Hudson and Hay [30].

Results and Discussion

It is evidenced from the results that the plant *Cassia alata* possesses strong antioxidant activity, as it contains good quantity of phenols (3.1 ± 0.67 µg/g), Vitamin-C (1.98 ± 0.47 µg/g), Vitamin- A (1.2 ± 0.22 µg/g), flavonoids (2.2 ± 0.51 µg/g), carotenoids (0.35 ± 0.50 µg/g) and anthraquinone (1.57 ± 0.36 µg/g) (Table 1).

In addition, it has strong DPPH radical scavenging activity (IC₅₀ = 54 ± 2.20), in contrast to the DPPH radical scavenging activity of a synthetic antioxidant compound, BHT (IC₅₀ = 72 ± 1.18) (Table 2).

Administration of aqueous leaf extract to the rabbits showed a noticeable increase in blood haemoglobin content, as well as in RBC count. There appeared a steep rising in the total count of leucocytes, with concomitant increasing in granulocyte: agranulocyte ratio. It is also evident from the results that the rabbit treated with the leaf extracts of *Cassia alata* resulted into a tremendous increase in the total number of peritoneal macrophages (Table 3). Thus, it is apparent from the results that aqueous leaf extract of *Cassia alata* plant has strong immune-modulating or immune-stimulating potency. During the course of experiment, abnormal behavior of the experimental animals

Conc. of Flavonoids* (µg/g)	Conc. of Vitamin-A* (µg/g)	Conc. of Vitamin-C* (µg/g)	Conc. of Anthraquinone* (µg/g)	Conc. of total Phenols* (µg/g)	Conc. of Carotenoids* (µg/g)
2.2 ± 0.51	1.2 ± 0.22	1.98 ± 0.47	1.57 ± 0.36	3.1 ± 0.67	0.35 ± 0.50

* Data are the mean value of five replicates.

Table 1: Antioxidant compounds present in the leaf tissue of *Cassia alata*.

Samples	Conc. of the sample	DPPH radical scavenging activity* [IC50 (g/ml ± S.D.)]
Leaf extract	100 µg/ml	54 ± 2.20
BHT	100 µg/ml	72 ± 1.18

* Data are the mean value of five replicates.

Table 2: DPPH Radical Scavenging activity of *C. alata* as compared to the synthetic antioxidant compound-BHT.

Properties	Before injection	After 7 days of injection	After 14 days of injection	After 21 days of injection
Body weight*	1.500	No change	1.525	1.550
Hemoglobin%* (g/100 ml blood)	9.2 g	No change	No change	9.4 g
Total WBC count* (per cu mm of blood)	4.800/cm ³	No change	No change	5.600/cm ³
Total RBC count* (per cu mm of blood)	3.7 million/cm ³	No change	No change	4.0 million/cm ³
Total Leucocyte count* (per cu mm of blood)	9.00 ± 0.20	No change	No change	17.00 ± 0.28
Granulocyte: A granulocyte ratio*	54.46 ± 0.48	No change	No change	60.42 ± 0.19
Total peritoneal macrophages count* (per cu mm of blood)	8 ± 0.11	No change	No change	25.0 ± 0.10

*Data are the mean values of five replicates.

Table 3: Assay of immune stimulating property of *Cassia alata*.

was not shown. Though there appeared an increase in eating habit, but there seemed to be no significant enhancement in their body weight.

An increase in these blood components is very significant, as they are essentially important parameters of body's defense system. Present study had revealed that the leaf extract of *Cassia alata* plant under consideration had significant impact on stimulation of host's immune system. It has been well established that macrophages play vital role for immune stimulation, tumor cell lysis and inhibition of tumor growth [31,32].

Cassia alata is one of the most important species of the genus *Cassia*, which is rich in anthraquinones and polyphenols. The leaves of *C. alata* have been qualitatively analyzed for the presence of primarily five pharmacologically active anthraquinones: rhein, aloemodin, chrysophanol, emodin and physcion, as well as the flavonoid, kaempferol [18]. The flavonoid, kaempferol has been reported to have anticancer properties [33].

Idu et al. [34] observed that preliminary phytochemical analysis of *Cassia alata* showed the presence of phenols, tannins, anthraquinones, saponins and flavonoids. Odunbaku and Lusanya [35] also confirmed that this plant also had alkaloids and cardenolides.

Sharma et al. [36] also reported in their study that Preliminary phytochemical screening of alcoholic extract revealed the presence of anthraquinone, phenolic compounds; saponin, glycosides, and while aqueous extract showed presence of glycosides and Phenolic compounds.

These anthraquinone derivatives are well known to exhibit a variety of biological activities, such as antibacterial and antifungal [37], antitumor [38], antioxidant [39], cytotoxic and hypoglycaemic activities [40,41]. Other chemicals contained in the plant include saponin, which acts as a laxative and expels intestinal parasites. Rhein and chrysophanol are also known to be present in the roots, in addition to two other quinone pigments [42,43].

Among the compounds obtained from the leaf extract of the plant *Cassia alata*, phenols and polyphenols have gained utmost importance due to their large array of bioactivities, including free radical scavenging, metal chelating, immune modulating activities, as well as modulation of enzyme activities, inhibition of lipid oxidation, etc. [44-46]. The term polyphenol refers to a complex group of compounds that contain in their structure an aromatic ring bearing one or more hydroxyl groups, which comprise simple phenols, such as phenolic acids and other derivatives of phenols, as well as some complex structures like flavones, flavonoids, anthocyanins, anthraquinones, etc.

Vitamin C (ascorbic acid) is an important component of our nutrients, and has been proved to exert a protective role against various stress related diseases, such as stroke, heart problems, cancer, and many neurodegenerative diseases [47]. And carotenoid, particularly, β -carotene is a vital antioxidant defense.

Presence of significant amount of phenols, anthraquinones, flavonoids, carotenoids, Vitamin-C and Vitamin-A in the methanolic leaf extract of *C. alata* indicates strong antioxidant properties of this plant.

Pieme et al. [48] investigated the acute to sub-acute toxicities of hydro-ethanolic extract of leaves of *Cassia alata* on Swiss mice and Wistar albino rat. However, the present study exhibited no cytotoxic effects of the aqueous leaf extract of *Cassia alata*. *Cassia alata* showed some protective effects on hepatocytes and improved liver architecture

[30]. These results showed that the use of the extract of *Cassia alata* is safe and explained the extensive utilization of the plant in traditional medicine. In the light of this, Chukeatirote et al. [49] stated that since ancient time, plant and animal products have been used for treatment of diseases and disorders, and that, plants in particular, have been used to treat infections due to its antimicrobial properties.

Conclusion

Today a lot of antioxidant products are available in the market. Though a burning topic of the day, plants need to be evaluated for their antioxidant potential, which will definitely be the right step towards the benefit of the mankind, keeping in view the present study, may certainly be a positive step towards green chemistry in the field of modern drug discovery, and this novel plant *Cassia alata* may extensively be used in therapeutic medicines as a resource of natural antioxidants and immune stimulating agent. However, further research is needed for its safety level and judicious use.

Acknowledgement

I wish to acknowledge UGC RFMS Scheme for financial support. I am also thankful to Mrs. Pampa Pal, Pubali Dev and my family for their help and inspiration throughout the work.

References

1. Gibbons E (1972) Stalking wild goods on a desert isle. National Geographic 142: 46-63.
2. Gibbons E (1973) Stalking the west's wild foods. National Geographic 144: 186-199.
3. Morton JF (1963) Principal wild food plants of the United States excluding Alaska and Hawaii. Economic Botany 47: 319-330.
4. Weiner MA (1972) Earth medicine and earth food. The Macmillian Company, NY, USA.
5. Chatterjee Sabyasachi, Chatterjee Saheli, Dutta Sikha (2013) *Cassia alata*-An useful antimicrobial agent. Medicinal and Aromatic Plants 2: e143.
6. Kumpulainen JT, Solonen JT (1999) Natural antioxidants and anticarcinogens in nutrition, health and disease. Royal Society of Chemistry UK, 187.
7. Cook NC, Samman S (1996) Flavonoids, chemistry, metabolism, cardio protective effects, and dietary sources. Nutr Biochem 7: 66-76.
8. Halliwell B (1994) Free radicals, antioxidants and human diseases curiosity, cause, or consequence. Lancet 344: 724.
9. Huhan J (1976) The flavonoids. A class of semi essential food components, their role in human nutrition. World Rev Nutr Diet 24: 117-191.
10. Younes M, Siegers CP (1981) Inhibitory action of some flavonoids on enhanced spontaneous lipid peroxidation following glutathione depletion. Planta Med 43: 240-245.
11. Schuler P (1990) Natural antioxidants exploited commercially. Food Antioxidants, Elsevier Applied Food Science Series 99-170.
12. Djeridane A, Yousfi M, Vidal N, Nadjemi, N, Lesgards JF, et al. (2007) Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. European Food Research and Technology 224: 801-809.
13. Kahkonen MP, Hopia AI, Ucorela HJ, Rauha JP, Pihlaja K, et al. (1999) Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 3954-3962.
14. Ibrahim D, Osman H (1995) Antimicrobial activity of *Cassia alata* from Malaysia. J Ethnopharmacol 45: 151-156.
15. Somchit MN, Reezal I, Elysha Nur IE, Mutalib AR (2003) *In vitro* antimicrobial activity of ethanol and water extracts of *Cassia alata*. J Ethnopharmacol 84: 1-4.
16. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2276639/>
17. Villaseñor IM, Canlas AP, Pascua MP, Sabando MN, Soliven LA (2002) Bioactivity studies on *Cassia alata* Linn. Leaf extracts. Phytother Res 16: 93-96.

18. Moriyama H, Iizuka T, Nagai M, Miyatake H, Satoh T (2003) Anti-inflammatory activity of heat-treated *Cassia alata* leaf extract and its flavonoid glycoside. *Yakugaku Zasshi* 123: 607-611.
19. Heyde H (1990) *Medicijn Planten in Suriname "Medicinal Plants in Suriname"*, Uitg. Stichting Gezondheidsplanten Informatie Paramaribo, 120.
20. Palanichamy S, Nagarajan S (1990) Analgesic activity of *Cassia alata* leaf extract and kaempferol 3-o-sophoroside. *J Ethnopharmacol* 29: 73-78.
21. Belkin M, Fitzgerald Dorothea B, Cogan W (1952) Tumor-damaging capacity of plant materials. I. Plants used as cathartics. *J Natl Cancer Inst* 13: 139-155.
22. Mahadevan A, Sridhar R (1986) *Methods in physiological plant pathology*. (3rd Edn), Sivakami Publications, India, 183-184.
23. Hertog MGL, Hollman PCH, Katan MB (1992) Content of potentially anti-carcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *J Agric Food Chem* 40: 2379-2383.
24. Oser BL (1979) *Hawks Physiological Chemistry*. McGraw Hill, New York, USA, 702-705.
25. Carr FH, Price EA (1926) Colour reactions attributed to vitamin A. *Biochem J* 20: 497-501.
26. Thimmaiah SK (1999) *Standard Methods of Biochemical Analysis*, Published by Kalyani Publishers, New Delhi, India, 304-305.
27. Panichayupakaranant P, Sakunpak A, Sakunphueak A (2009) Quantitative HPLC determination and extraction of anthraquinones in *Senna alata* leaves. *J Chromatogr Sci* 47: 197-200.
28. Sakulpanich A, Gritsanapan W (2009) Determination of anthraquinone glycoside content in *Cassia fistula* leaf extracts for alternative source of laxative drug. *International Journal of Biomedical and Pharmaceutical Sciences* 3: 42-45.
29. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und-Technologie/ Food Science and Technology* 28: 25-30.
30. Hudson L, Hay FC (1980) *Practical Immunology*. Blackwell Scientific, Oxford, London, UK.
31. Pradeep NS, Evans DA, Kumuthakalavalli R, Abraham TK (2005) Effect of *Pleurotus* spp. in basic immunological parameters in mice. *J Mycopath Res* 43: 57-61.
32. Cristina, Harry L, Wichers JH, Savelkoul FJ (2005) Mediators of Inflammation 2: 63-80.
33. Fernand VE, Dinh DT, Washington SJ, Fakayode SO, Losso JN, et al. (2008) Determination of pharmacologically active compounds in root extracts of *Cassia alata* L. by use of high performance liquid chromatography. *Talanta* 74: 896-902.
34. Idu M, Omonigho SE, Igeleke CL (2007) Preliminary investigation on the phytochemistry and antimicrobial activity of *Senna alata* L. flower. *Pak J Biol Sci* 10: 806-809.
35. Odunbaku OA, Lusanya OAF (2011) Synergistic effect of ethanol leaf extract of *Senna alata* and antimicrobial drugs on some pathogenic microbes. *Adv Environ Biol* 5: 2162-2165.
36. Sharma S, Dangi MS, Wadhwa S, Daniel V, Tiwari A (2010) Antibacterial activity of *Cassia tora* leaves. *Int J Pharm Biol Arch* 1: 84-86.
37. Chatterjee S, Chatterjee S, Dutta S (2010) A survey on VAM association in three different species of *Cassia* and determination of antimicrobial property of these phytoextracts. *J Med Plants Res* 4: 286-292.
38. Koyama J, Morita I, Tagahara K, Ogata M, Mukainaka T, et al. (2001) Inhibitory effects of anthraquinones and bianthraquinones on Epstein-Barr virus activation. *Cancer Lett* 170: 15-18.
39. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2276639/>
40. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2276639/>
41. Choi SB, Ko BS, Park SK, Jang JS, Park S (2006) Insulin sensitizing and alpha-glucosidase inhibitory action of sennosides, rheins and rhaponticin in *Rheirhizoma*. *Life Sci* 78: 934-942.
42. Co LL (1989) *Common Medicinal Plants of the Cordillera Region*. Bustamante Press; Quezon City, Philippines 11.
43. Tiwari RD, Yadava OP (1971) Structural study of the quinone pigments from the roots of *Cassia alata*. *Planta Med* 19: 299-305.
44. Teissedre P, Landrault N (2000) Wine phenolics: Contribution to dietary intake and Bioavailability. *Food Res Int* 33: 461-467.
45. Mallavadhani U, Sudhakar A, Sathyanarayana KVS, Mahapatra AWLi, Richard B (2006) Chemical and analytical screening of some edible mushrooms. *Food Chem* 95: 58-64.
46. Rodrigo R, Bosco C (2006) Oxidative stress and protective effects of polyphenols: Comparative studies in human and rodent kidney. *Comp Biochem Physiol C Toxicol Pharmacol* 142: 317-327.
47. Halliwell B (1996) Vitamin C. An antioxidant or Pro-oxidant *in vivo*. *Free Radic Res* 25: 439-454.
48. Pieme CA, Penlap VN, Nkegoum B, Taziebou CL, Tekwu EM, et al. (2006) Evaluation of acute and subacute toxicities of aqueous ethanolic extract of leaves of *Senna alata* (L.) Roxb (*Cesalpinaceae*). *Afr J Biotechnol* 5: 283-289.
49. Chukeatirote E, Hanpattanakit P, Kaprom A, Tovarantonont J (2007) Antimicrobial activity of *Senna spectabilis* and *S. tora*. *J Plant Sci* 2: 123-126.