Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 4, Issue 3, 2012

Research Article

# COMPARATIVE ANALYSIS OF ANTIOXIDANT ACTIVITY AND PHYTOCHEMICAL SCREENING OF SOME INDIAN MEDICINAL PLANTS

**UMESH KUMAR* AND VEERU PRAKASH**

**Department of Biochemistry and Bioprocess Technology, Jacob School of Biotechnology and Bioengineering, Sam Higginbottom Institute of Agriculture, Technology and Sciences (Deemed to be University), Allahabad, UP India- 211007. Email: umesh.biochem@gmail.com**

*Received: 18 Feb 2012, Revised and Accepted: 23 Mar 2012*

**ABSTRACT**

Indian medicinal plants (*Casia fistula*, *Cinnamomum cassia*, *Acacia catechu* and *Citrus limon*) were analyzed for their antioxidant activity, phytochemical composition and vitamins content. Methanolic extracts of the bark of these plant species showed high antioxidant activity and *Casia fistula* possessed the highest percent inhibition of DPPH (91.66%) amongst all studied plants. The results also revealed the presence of substantial amount of bioactive constituents comprising alkaloids (1.31 to 1.64 mg/100g DW), flavonoids (36.2 to 76.2 mg/100g DW), saponins (0.883 to 2.251 mg/100g DW), tannins (0.45 to 0.85 mg/100g DW) and total phenol content (110 to 210.2 mg/100g DW) where *Casia fistula* was observed comparatively richer source of these phytochemicals. The medicinal plants had the erratic concentrations of vitamins and contained carotenoids (104 to 135 mg/100g DW), ascorbic acid (18.05 to 55.04 mg/100g DW), thiamine (0.12 to 0.28 mg/100g DW), riboflavin (0.11 to 0.42 mg/100g DW) and niacin (0.02 to 0.08 mg/100g DW). The results provided the evidence that the studied medicinal plants are to be potent source of natural antioxidant and medicinally important compounds.

**Keywords:** Antioxidant activity, Bioactive compounds, Ethnomedicine, Medicinal plants, Vitamins.

## INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Medicinal plants typically contain mixtures of different chemical compounds that may act individually, additively or in synergy to improve health. The main objective of this study is, to search for some Indian plants with strong antioxidant activity which could serve as good candidates for the development of standardized phytomedicine.

Oxidative stress is one of the most important routs for producing free radicals in food, drugs and even living systems. Environmental pollutants e.g. radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Free radicals contribute to more than one hundred disorders in human beings including atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS[1]. Antioxidants can be effective in preventing free radical formation by scavenging them or promoting their decomposition and up pressing such disorders[2]. Currently, there is a growing interest toward natural antioxidants of herbal resources. Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly supported this idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems[3].

*Acacia catechu* is a sacred plant that belongs to the family Mimosaceae. It contains a number of chemical constituent that interact in a complex way to elicit their pharmacodynamic responses. It is highly effective in a wide spectrum of diseases and reported to posses antibacterial, antifungal, anti-inflammatory and antioxidant properties[4]. It also helps in clotting of blood in case of any injuries.

*Cassia fistula* Linn. (Family- Leguminosae) is of great therapeutic value in treating diseases such as liver disorder, gout, dysentery, leprosy and diabetes. It also helps in shrinking engorged veins and has a powerful antioxidant and anti- inflammatory effect[5]. It has the right balance of lipids, proteins and carbohydrate hence it is very much effective in providing strength to the body.

*Cinnamomum cassia* (Family- Lauraceae) bark is attributed with numerous medicinal uses such as diaphoretic, antipyretic and analgesic. It is used as a carminative, cardiac stimulant, antioxidant, refrigerant and diuretic[6]. It stimulates the nervous system of our body. It works as good appetizer and improves digestion. It also stimulates liver for proper functioning and it is also very effective treatment in tuberculosis due to presence of sinemic acid.

*Citrus limon* (Family- Rutaceae) its therapeutic effects have been attributed to its vitamin C rich fruit pulp. The seeds and bark are used in the treatment of asthma, bronchitis, scurvy, rheumatism, dysentery and diarrhea, it also posses bactericidal and antioxidant properties. It improves circulation of the blood and is helpful in condition like atherosclerosis.

In particular, despite widespread use of aromatic plants as medicines in India, the literature contains few reports of antioxidant activity and chemical composition of these plants. In present study, we carried out a systematic record of the relative free radical scavenging activity in selected Indian medicinal plant species.

## MATERIAL AND METHODS

### Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, tert-butyl-4-hydroxy toluene (BHT), Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany).

### Plant materials

The four medicinal plants studied were collected from Auraon Research Center (an authenticated research center recognized by Central Scientific and Industrial Research, India), Botanical garden of National Botanical Research Institute, Lucknow. The plant materials were cleaned and powdered. The botanical names, family names, English names and parts used are presented in Table 1.

**Table 1: Brief introduction of medicinal plants**

| Botanical Nameused | English Name | Part | Family | Medicinal use |
|---|---|---|---|---|
| Acacia catechu L.antifungal | Mimosa catechu | Bark | Fabaceae | Antibacterial, |
| Cassia fistula L.treeinflammatory | Golden shower | Bark | Fabaceae | Antioxidant, anti- |
| Cinammomum cassia L.antipyretic, analgesic | Chinese cinnamon | Bark | Lauraceae | Diaphoretic, |
| Citrus limon L.antioxidant | Lemon | Bark | Rutaceae | Bactericidal, |

**Extraction**

The plant materials presented in Table 1 were air-dried in shed at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. Methanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 1 litre of methanol at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. The percentage yield of extracts ranged from 5 - 20% (w/w).

**Antioxidant activity (DPPH free radical scavenging activity) determination**

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity by the method given by Yen and Duh[7]. Ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. BHT and quercetin were used as standard controls. $IC_{50}$ values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation.

Percent (%) inhibition of DPPH activity = [(AB – AA) / AB] x 100

Where AA and AB are the absorbance values of the test and of the blank samples respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as $IC_{50}$ value for each of the test solutions.

**Determination of alkaloids**

5 g of the sample were weighed into a 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed[8].

**Determination of Flavonoid**

10 g of the plant samples were extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper no. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water bath and weighed[9].

**Determination of Saponin**

20 g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separator funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight[8].

**Determination of Tannins**

Weigh 0.5 g of powdered material and transfer to a 250ml of conical flask. Add 75ml of distilled water. Heat the flak gently and boil for 30 min. centrifuge at 2000rpm for 20 min and collect the supernatant in 100 ml of volumetric flask and make up the volume. Transfer 1 ml of the sample extract to a 100 ml volumetric flask containing 75 ml distilled water add 5 ml of Folin-Denis reagent, 10 ml of $Na_2CO_3$ and dilute to 100 ml with distilled water. Shake well and read the absorbance at 700 nm after 30 min. Prepare a blank with water instead of sample. Prepare the standard graph by using standard tannic acid[10].

**Determination of total phenol**

Total phenols were determined by Folin Ciocalteu reagent[11]. A dilute extract of each plant extract (0.5 ml of 1:10 g $ml^{-1}$) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous $Na_2CO_3$ (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg $L^{-1}$ solutions of gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg $g^{-1}$ of dry weight), which is a common reference compound.

**Determination of carotenoids**

Total carotenoids were determined by the method of Jensen[12]. One gram sample was extracted with 100 ml of 80% methanol solution and centrifuged at 4000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic KOH the mixture was washed with 5% ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.

**Determination of ascorbic acid**

The ascorbic acid was determined according to Cakmak and Marschner[13] with some modification. Each plant extract (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 5 ml of 5% meta-phosphoric acid, and centrifuged at 4000 rpm for 30 min. The reaction mixture contained 0.2 ml aliquot of the 4000 rpm supernatant, 0.5 ml 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.1 ml (10 mM) DTT (1,4-dithiothreitol) and 0.1 ml (0.5%, w/v) N-ethylmaleimide (NEM) to remove excess DTT. The color was developed after addition of the following reagents in the reaction mixture: 0.4 ml (10%) trichloroacetic acid (TCA), 0.4 ml (44%) ortho-phosphoric acid, 0.4 ml (4%) 2, 2-bipyridine in 70% ethyl alcohol, and 0.2 ml (3%) $FeCl_3$. The mixture was then incubated at 40°C for 40 min, and the absorbance was measured at 525 nm. Ascorbic acid was used as a standard in the range of 0 to 100 µg/ml.

**Determination of thiamine**

5 g of the sample were homogenized with ethanolic sodium hydroxide (50 ml). It was filtered into a 100 ml flask. 10 ml of the filtrate was pipette and the colour developed by addition of 10 ml of potassium dichromate and read at 360 nm. A blank sample was prepared and the color also developed and read at the same wavelength[14].

**Determination of riboflavin**

5 g of the sample was extracted with 100 ml of 50% ethanol solution and shaken for 1 h. This was filtered into a 100 ml flask; 10 ml of the extract was pipette into 50 ml volumetric flask. 10 ml of 5% potassium permanganate and 10 ml of 30% $H_2O_2$ were added and allowed to stand over a hot water bath for about 30 min. 2 ml of 40% sodium sulphate was added. This was made up to 50 ml mark and the absorbance measured at 510 nm in a spectrophotometer[15].

**Determination of niacin**

5 g of the sample was treated with 50 ml of 1 N sulphuric acid and shaken for 30 min. 3 drops of ammonia solution were added to the sample and filtered. 10 ml of the filtrate was pipette into a 50 ml volumetric flask and 5 ml potassium cyanide was added. This was acidified with 5 ml of 0.02 N $H_2SO_4$ and absorbance measured in the spectrophotometer at 470 nm wavelengths[16].

**RESULTS**

Preliminary phytochemical screening of the studied plants (*Cassia fistula*, *Cinnamomum cassia*, *Acacia catechu*, and *Citrus limon*) revealed the presence of phytochemicals: alkaloids, saponins, flavonoids, tannins, total phenol, carotenoids, ascorbic acid, thiamine, riboflavin and niacin.

Among all four medicinal plants used in the study, the maximum antioxidant activity 91.66 ± 4.33 (DPPH percentage inhibition) was observed in *Cassia fistula* followed by *Cinnamomum cassia (*86.6 ± 4.33 mg/100g DW), *Acacia catechu (*76 ± 1.66 mg/100g DW) and the least activity was noted in *Citrus limon* (67.33 ± 3.33 mg/100g DW) (Table 2).

**Table 2: Antioxidant activity of *Casia fistula*, *Acacia catechu*, *Cinnamomum cassia*, and *Citrus limon*.**

| Plants name | Antioxidant activity (%DW) |
|---|---|
| *Cassia fistula* | 91.66 ± 4.33 |
| *Acacia catechu* | 76 ± 1.66 |
| *Cinnamomum cassia* | 86.66 ± 4.33 |
| *Citrus limon* | 67.33 ± 3.33 |

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

The variable concentrations of alkaloids were observed in the bark of studied plants. The *Cinnamomum cassia* showed highest value (1.64 ± 0.22 mg/100g DW) as compared to other three medicinal plants and lowest source was examined to be *Citrus limon* (1.31 ± 0.16 mg/100g DW). *Citrus limon* and *Acacia catechu* both had almost similar concentrations of alkaloid but *Cassia fistula* possessed some higher content and reported to be 1.54 ± 0.15 mg/ 100g DW. Analysis of bark samples showed *Cassia fistula* is the richest source of flavonoids (76.2 ± 4.5 mg/100g DW) which was found almost two fold higher than *Acacia catechu* (36.2 ± 1.2 mg/100g DW). *Cinnamomum cassia* and *Citrus limon* had better content of flavonoids than *Acacia catechu* and possessed 59.4 ± 5.4 and 48.3 ± 1.3 mg/100g DW respectively (Table 3). Similarly, Saponins content was seen significantly elevated in *Cassia fistula* (2.251 ± 0.020mg/100g DW) while *Citrus limon (*0.883 ± 0.004mg/100g DW*)* was recorded to be poorest source in the studied plants. *Acacia catechu* contained a bit higher concentration of saponins (1.605 ± 0.012 mg/100g DW) than *Cinnamomum cassia* (1.115 ± 0.014 mg/100g DW) but contained double amount than *Citrus limon*.

Tannins were present in trace amount with their highest concentration (0.85 ± 0.26 mg/1000g DW) found in *Cinnamomum cassia,* near about same content was obtained from *Cassia fistula* (0.78 ± 0.20 mg/100g DW). *Acacia catechu* (0.52 ± 0.15 mg/100g DW) had more tannins than *Citrus limon* (0.45 ± 0.22 mg/100g DW) which had the least amount. Total phenol was found as the major constituent of these medicinal plants. Values recorded in the table-3 reveals that *Cassia fistula* and *Cinnamomum cassia* possessed significantly higher total phenol and contained 210.2 ± 3.2 and 198.0 ± 4.2 mg/100g DW respectively. The bark of *Acacia catechu* (156.3 ± 2.8 mg/100g DW) was also seen to be better storage of total phenol while its minimum concentration was obtained from *Citrus limon* (110.0 ± 3.1 mg/100g DW) (Table 3).

**Table 3: Phytochemical constituent of Indian medicinal plants expressed as mg/100g dry weight (DW).**

| Plant name | Flavanoid(mg/100g DW) | Total phenol (mg/100g DW) | Alkaloids (mg/100g DW) | Tannins (mg/100g DW) | Saponin (mg/100g DW) |
|---|---|---|---|---|---|
| *Casia fistula* | 76.2 ± 4.5 | 210.2 ± 3.2 | 1.54 ± 0.15 | 0.78 ± 0.20 | 2.251 ± 0.02 |
| *Acacia catechu* | 36.2 ± 1.2 | 156.3 ± 2.8 | 1.36 ± 0.11 | 0.52 ± 0.15 | 1.605 ± 0.012 |
| *Cinnamomum cassia* | 59.4 ± 5.4 | 198 ± 4.2 | 1.64 ± 0.22 | 0.85 ± 0.26 | 1.115 ± 0.014 |
| *Citrus limon* | 48.3 ± 1.3 | 110 ± 3.1 | 1.31 ± 0.16 | 0.45 ± 0.22 | 0.883 ± 0.004 |

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

Results of analysis of *Cassia fistula*, *Acacia catechu*, *Cinnamomum cassia* and *Citrus limon* showed that these plants are also a rich source of vitamins. The concentration of carotenoids was recorded maximum in *Cassia fistula* (135 ± 15 mg/ 100g DW) and minimum in *Citrus limon* (104 ± 1.66 mg/100g DW) whereas *Acacia catechu* had more carotenoids as compared to *Cinnamomum cassia* (Table 4). Among all the four plants, *Citrus limon* (55.04 ± 0.23 mg/ 100g DW) was found to be the prosperous source of ascorbic acid and contained two and half folds more ascorbic acid than *Cinnamomum cassia* whereas three folds than *Acacia catechu*. Concentration of ascorbic acid in *Casia fistula, Cinnamomum cassia and Acacia catechu* was reported to be 40.05 ± 0.18, 22.51 ± 0.28 and 18.05 ± 0.32 mg/ 100g DW respectively. A significantly highest concentration of thiamine (0.28 ± 0.42 mg/ 100 g DW) was obtained in *Cinnamomum cassia* while lowest (0.12 ± 0.26 mg/ 100 g DW) in *Citrus limon.* Its concentration was 0.22 ± 0.38 mg/ 100 g DW in *Casia fistula* and 0.14 ± 0.20 mg/ 100g DW in *Acacia catechu* (Table 4).

Maximum riboflavin concentration (0.42 ± 0.10 mg/ 100g DW) was detected in *Cassia fistula.* Next to it was *Cinnamomum cassia* (0.21 ± 0.09 mg/100g DW) and *Acacia catechu* (0.18 ± 0.14 mg/100g DW) whereas least amount was obtained from *Citrus limon* (0.11 ± 0.22 mg/100g DW). Concentration of niacin was found maximum in medicinal plant *Cinnamomum cassia* (0.07 ± 0.14 mg/100g DW) very much close to *Casia fistula* (0.08 ± 0.12 mg/100g DW), also *Citrus limon* (0.04 ± 0.20 mg/100g DW) had about similar concentration to *Acacia catechu* (0.02 ± 0.11 mg/100g DW) that had least niacin content (Table 4).

**Table 4: Vitamins composition of Indian medicinal plants expressed as mg/100g dry weight (DW).**

| Plant name | Ascorbic acid(mg/100g DW) | Riboflavin (mg/100g DW) | Thiamine (mg/100g DW) | Niacin (mg/100g DW) | Carotenoids (mg/100g DW) |
|---|---|---|---|---|---|
| *Casia fistula* | 40.05 ± 0.18 | 0.42 ± 0.10 | 0.22 ± 0.38 | 0.08 ± 0.12 | 135 ± 15 |
| *Acacia catechu* | 18.05 ± 0.32 | 0.18 ± 0.14 | 0.14 ± 0.20 | 0.02 ± 0.11 | 123.6 ± 4.33 |
| *Cinnamomum cassia* | 22.51 ± 0.28 | 0.21 ± 0.09 | 0.28 ± 0.42 | 0.07 ± 0.14 | 118 ± 0.66 |
| *Citrus limon* | 55.04 ± 0.23 | 0.11 ± 0.22 | 0.12 ± 0.26 | 0.04 ± 0.20 | 104 ± 1.66 |

All values are mean ± standard deviation (n = 3). Means with different letters within a column are significantly different (P < 0.05).

**DISCUSSION**

Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural population depends on it as primary health care[17].

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts[18]. A strong correlation has been observed between the phenols and

antioxidant activity. Also strong relationship between total phenolics content and antioxidant activity has been reported by Kahkonen et al.[19]. In contrast, relationship was not observed between the flavonoids content and antioxidant activity. The results of the present study are in agreement with that of Miliauskas *et al.* and Gracia- Alonso *et al.*[20, 21].

The secondary metabolite constituents of *Cassia fistula*, *Acacia catechu*, *Cinnamomum cassia* and *Citrus limon* detected include the alkaloids, flavonoids, saponins and tannins. Pure isolated alkaloids and their synthetic derivatives are used as basic medicinal agents for their analgesic, antispasmodic and bactericidal effects[22]. The presence of alkaloids in these plants is also used for hypertension treatment[23]. Flavonoids, are potent water-soluble antioxidants and free radical scavengers[24], which prevent oxidative cell damage, have strong anticancer activity[25]. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity[25].

The high saponin content of *Cassia fistula*, *Acacia catechu*, *Cinnamomum casia* and *Citrus limon* justifies the use of the extracts from these plants to stop bleeding and in treating wounds. Saponin has the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness[25, 26]. Saponins prevent the excessive intestinal absorption of this cholesterol and thus reduce the risk of cardiovascular diseases such as hypertension[27]. Tannins have stringent properties, hasten the healing of wounds and inflamed mucous membranes[28]. Recent studies have shown that, many polyphenol compounds contribute significantly to the total antioxidant activity of many plants[29]. The total phenols contain good antioxidant, antimutagenic and anticancer properties[30]. *Cassia fistula and Cinnamomum cassia* had high total phenol content while the highest concentration of total phenols responsible for the antioxidant activity.

Carotenoids play an important role in plant reproduction: along with phenolics they are responsible for bright colors. This chemical class acts as antioxidants, with functions that include protection of membranes against damage by free radicals and retardation of ageing processes[31]. It act as photo protective agents and may reduce the risk of sunburns, photo-allergy and even some types of skin cancer[32]. The examined result shows that *C. fistula and A. catechu* are strong source of carotenoids and it can be a promising plant for use in pharmacological products designed for antioxidant activity.

Ascorbic acid is a powerful reducing agent capable of rapidly scavenging a number of reactive oxygen species. L-Ascorbate (Vitamin C) is considered as the strong reducing agent carrying out the reducing function which may reduce the risk of chronic diseases such as cancer, cardiovascular and helps make collagen, a tissue needed for gums and blood vessels[33]. As a result of the availability of ascorbic acid in *C. fistula, A. catechu, C. cassia* and *C. limon* these plants are used in herbal medicine for the treatment of common cold and other diseases like prostrate cancer[25]. The most important role of thiamin is to treat beri beri and Wernicke-Korsakoff syndrome, it is a brain disorder caused by thiamine deficiency[34]. Thiamin, Riboflavin and Niacin also play most important role in Cataract[35], Heart failure[36] and Alzheimer's disease[37]. *Cassia fistula and Acacia catechu* use as an herbal medicine and further drug formulation for heart failure and Alzheimer's disease.

## CONCLUSION

This present study has revealed that the antioxidant activity, phytochemicals and vitamins composition of *Cassia fistula*, *Acacia catechu*, *Cinnamomum cassia* and *Citrus limon* in bark extracts. This study showed the antioxidant properties of medicinal plants playing part significantly in anti-inflammatory, heart failure and carbohydrate metabolism disorders. The investigation supports the traditional use of these plants in postpartum care. Some medicinal plants are potent antioxidants and may be efficient as preventive agents in many diseases. This partly shows the use of phytochemicals, coupled with the presence of the essential vitamins. These plants can be seen as a potential source of useful food and drugs. Further studies have to be carried out to isolate, characterize and elucidate the structure of the bioactive compounds from the plants for the industrial drug formulation.

## ACKNOWLEDGEMENT

We are thankful to Hon'ble Vice-chancellor of Sam Higginbottom Institute of Agriculture, Technology & Sciences, Deemed- to-be University, Allahabad for providing all necessary facilities to conduct the experiment.

## REFERENCES

1. Kumpulainen JT, Salonen JT, Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease, the Royal Society of Chemistry, UK, 1999. pp. 178- 187.
2. Halliwell B, The antioxidant paradox. Lancet 2000; 355: 1179-1180.
3. Ness AR, Powles JW, A review: Fruit and vegetables and cardiovascular disease. Int J Epidemiol 1997; 26: 1-13.
4. Burnett BP, Jia Q, Zhao Y, Levy RM, A medicinal extract of *Scuetellaria baicalensis* and *Acacia catechu* acts as a dual inhibitor of cyclooxygenase and 5-lipoxygenase to reduce inflammation. J Med Food 2007; 10(3): 442-451.
5. Ilavarasan R, Mallika M, Venkataraman S, Anti-inflammatory and antioxidant activities of *Casia fistula* Linn. bark extracts. Afr J Tred CAM 2005; 2 (1): 70-85.
6. Masood N, Chaudhry A, Tariq P, Antimicrobial activity of *Cinnamomum cassia* against diverse microbial flora with its nutritional and medicinal impacts. Pak J Bot 2006; 38(1): 169-174.
7. Yen GC, Duh PD, Scavenging effect of methanolic extracts of peanut hulls on free-radical and active oxygen species. J Agric Food Chem 1994; 42: 629-632.
8. Obadoni BO, Ochuko PO, Phytochemical studies and Comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Global J Pure Appl Sci 2001; 8: 203-208.
9. Boham AB, Kocipai AC, Flavonoid and condensed tannins from Leaves of Hawaiian vaccininum vaticulum and vicalycinium. Pacific Sci. 1994; 48: 458-463.
10. Schanderi SH, In: Methods in food analysis, Academic Press, NewYork, 1970; pp. 709.
11. Ragazzi E, Veronese G, Quantitative analysis of phenolic compounds after thin layer chromatographic separation. J Chromatogr 1973; 77: 369-375.
12. Jensen A, Chlorophyll and carotenoids. In: Hallebust JA, Craigie JS. (eds). Handbook of Physiochemical and Biochemical Methods. Cambridge University Press, Cambridge, UK, 1978; pp. 5-70.
13. Cakmak I, Marschner H, Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase in bean leaves. Plant Physiol 1992; 98: 1222-1227.
14. Sadashivam S, Manickam A, In: Biochemical Methods for Agricultural Sciences, Willy Eastern Limited, New Delhi, 1992; pp. 173-175.
15. Hodson AZ, Norris LC, Determination of riboflavin in biological material by means of photoelectric colorimetry. J Biol Chem 1931; 131: 621.
16. Melnick D, Field HJr, Determination of nicotinic acid in biological material by means of photoelectric colorimetry. J Biol Chem 1940; 134: 1-2.
17. World Health Organization: The promotion and development of traditional medicine. Technical report series, WHO, Janeva, 1978; pp. 622.
18. Koleva II, Van-Beek TA, Linssen JPH, De-Groot A, Evstatieva LN, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem Analy 2002; 13: 8-17.
19. Kahkonen MP, Hopia AT, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al., Antioxidant activity of plant extracts containing phenolic compounds. J Agricul Food Chem 1999; 47: 3954 – 3962.
20. Miliauskas G, Venskutonis PR, Van-Beek TA, Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004; 85: 231- 237.

21. Garcia-Alonso M, Pascual-Teresade S, Santos-Buelga C, Rivas-Gonzalo JC, Evalution of the antioxidant properties of fruit. Food Chem 2004; 84: 13- 18.
22. Okwu DE, Okwu ME, Chemical composition of *Spondias mombin* L. plant parts. J Sustain Agricul Environ 2004; 6(2): 140-147.
23. Olaleye MT, Cytotoxicity and antibacterial activity of Methanolic extract of *Hibiscus sabdariffa.* J Med Plants Res 2007; 1(1): 9-13.
24. Stauth D, Studies force new view on biology of flavonoids. Oregon State University.USA, 2007.
25. Okwu DE, Phytochemical and vitamin content of indigenous spices of southeastern Nigeria. J Sustain Agricul Environ 2004; 6(1): 30-37.
26. Sodipo OA, Akiniyi JA, Ogunbamosu JU, Studies on certain characteristics of extracts of bark of pansinytalia macruceras pierre Exbeille. Glo J Pure Appl Sci 2000; 6: 83-87.
27. Akinpelu DA, Onakoya TM, Antimicrobial activities of medicinal plants used in folklore remedies in south-western. Afri J Biotechnol 2006; 5: 1078-1081.
28. Okwu DE, Josiah C, Evaluation of the chemical composition of two Nigerian medicinal plants. Afri J Biotechnol 2006; 5 (4): 357-361.
29. Luo XD, Basile MJ, Kennelly EJ, Polyphenolic antioxidants from the fruits of *Chrysophyllum cainito* L. (Star apple). J Agric Food Chem 2002; 50: 1379-1382.
30. Ahmad N, Mukhtar H, Green tea polyphenols and cancer: biological mechanisms and practical implications. Nutr Rev 1999; 57(3): 78-83.
31. Bulda O, Rassadina V, Alekseichuk H, Laman N, Spectrophotometric measurement of carotenes, xanthophylls, and chlorophylls in extracts from plant seeds. Russ J Plant Physiol 2008; 55(4): 544-551.
32. Lee J, Jiang S, Levine N, Watson R, Carotenoid supplementation reduces erythema in human skin after simulated solar radiation exposure. Proc Soc Exp Biol Med 2000; 223: 170-174.
33. Singh P, Singh U, Shukla M, Singh RL, Variation of some phytochemicals in *Methi* and *Saunt* plants at different stages of development. J Herbal Med Toxici 2000; 4(2): 93-99.
34. Bonucchi J, Hassan I, Policeni B, Kaboli P, Thyrotoxicosis associated with Wernicke's encephalopathy. J Gen Intern Med 2008; 23(1): 106-109.
35. Kuzniarz M, Mitchell P, Cumming RG, Flood VM, Use of vitamin supplements and cataract: The Blue Mountains Eye Study. Am J Ophthalmol 2001; 132(1): 19-26.
36. Sica DA, Loop diuretic therapy, thiamine balance, and heart failure. Cong Heart Fail 2007; 13(4): 244-247.
37. Rodriquez-Martin JL, Qizilbash N, Lopez-Arrieta JM, Thiamine for Alzheimer's disease (Cochrane Review). Cochr Data Syst Rev 2001; 2: 1498.