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# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT AND DIFFERENT FRACTIONS OF *CASSIA ANGUSTIFOLIA* ROOTS

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## ABSTRACT

The present study was conducted to investigate the phytochemical composition and antioxidation activities of methanolic extract and its different solvent fractions. The Phytochemical analysis was carried out to evaluate the presence of alkaloids, flavonoids, saponins, terpenoids, anthraquinones, coumarins, and tannins. Quantitative analysis of total phenolic contents(TPC) and total flavonoids contents(TFC) of extracts were measured by Folin-Ciocalteu and Aluminium chloride methods respectively. Among all sample extract fractions, ethyl acetate fraction (CA-EA) was found to contain highest amount of phenols (199.24 ± 1.25 mg GAE/g) and maximum concentration flavonoid contents (153.45 ± 1.76 mg RU/g) were found in sample extract. The obtained results showed that CA-EA fraction possess strong antioxidation activity in both DPPH and ABTSassays with lowest $IC_{50}$ value of 50.63 ± 1.23 and 55.04 ± 2.56 µg/mL respectively. However water fraction (CA-water) exhibited weakest antioxidation activity in both invitro assay methods, manifested from its higher $IC_{50}$ values. From over all data, it was observed that strong antioxidation capability of plants extracts depends upon the presence polyphenol contents.

**Keywords: *Cassia angustifolia,* phytochemical analysis, total phenol and flavonoid contents, antioxidation activities**

## INTRODUCTION

Free radicals, which are formed as a result of various metabolic reactions in human body may produce the hazardous effects on human health [1]. Most common free radicals are reactive oxygen species (ROS) which inhibit the normal cellular functions of proteins, lipids, DNA thereby causing the damage of cells [2]. In order to maintain the normal health of livings, there must be equilibrium between production and depletion of ROS by the natural defence system of living organisms [3]. Naturally in human body antioxidative enzymes are present which encounter these radical and make them neutral. Rancidity of free fatty acids in food is due to process of oxidation mediated by free radicals [4]. Antioxidants are the secondary metabolites of plant origin, which are mostly polyphenolic compounds, having ability to scavenge free radicals, thereby preventing oxidation reaction [5]. In nature, medicinal plants have ample amount of phenolic compounds which perform various biological effects, one of them is to counter oxidative stress caused by free radicals [6]. Phytochemicals are the plant's metabolites synthesized by various biochemical reactions in different parts of plants. They are used to cure disease in herbal and homeopathic medicines. Secondary metabolites such as phenols, tannins, saponins, alkaloids, steroids, flavonoids are responsible for different biological activities of plant extracts [7, 8].

*Cassia angustifolia* Vahl. is commonly known as Indiansenna, which belongs to family Caesalpiniaceae. This plant attains height of 5–8 meters and cultivated in tropical countries. The decoction of its leaves has been used asantihelmentics, antipyretic and laxative [9].Phytochemicaly leaves of Senna plant are found to contain, emodin, chrysophanol, anthraquinones glycosides including sennoside A, B, C D. Apart from this kamepferol, isorhamnetin flavonoids are also reported from this plant [10].

## MATERIALS AND METHODS

**Chemicals:** All the solvents and chemicals used were of analytical grade. Gallic acid, Folin-ciocalteu reagent, 2,2- Diphenyl-1-picrylhydrazyl (DPPH), Ascorbic acid, Aluminium chloride, Sodium carbonate, Ferric chloride, Potassium acetate and Rutin, were obtained from Sigma Chemical Co.

**Collection of plant material:** The roots of *Cassia angustifolia* Vahl (*C. angustifolia*) were collected from Jamshoro, Sindh, Pakistan, identified by one our author. Roots were washed and dried carefully under shade. The sample specimen was deposited in Department of

Pharmacognosy, University of Sindh Jamshoro. Dried roots were chopped to make small pieces.

**Preparation of plant extract:** Dried roots of *C. angustifolia*(500 g) were extracted with methanol (1L × 3, 4hrs) by sonication at room temperature and filtered, which was evaporated using vacuum rotary evaporator to obtain 56 g of dried extract, which was suspended in 300ml of water and was successively partitioned between equal volume of n-hexane, ethyl acetate, and n-butanol to yield 8.5g of CA-Hex , 10.6g of CA-EA, 16.8g of CA-BuOH and 25.6 g of CA-water fraction, after drying them through rotary vacuum evaporator.

**Phytochemical screening**

**Test for alkaloids:** 200mg of CA-MeOH extract was stirred with 6 ml of 1% HCl and the mixture was heated and filtered, later 3 mL of filtrate was reacted with few drops of potassium mercuric iodide (Mayer's reagent) Turbidity or formation of precipitates indicates the existence of alkaloids[11].

**Test for flavonoids:** 50 mg of CA-MeOH extract was suspended in 50 mL of distilled water and filtered. 4 mL of dilute ammonia solution was added to 8 mL of filtrate followed by few drops of concentrated $H_2SO_4$. Formation of yellow color indicates the presence of flavonoids [11].

**Test for saponins:** 20 mg of CA-MeOH extract was poured in 25 mL of distilled water on a water bath for 10 minutes and filtered. 4 mL of the filtrate was mixed with 4 mL of distilled water and shaken for froth formation. 4 drops of olive oil were poured in froth and shaken vigorously to observe the formation of emulsion [11].

**Test for terpenoids:** CA-MeOH extract (5mg/ml) was mixed with 4 ml of chloroform, followed by few drops of concentrated $H_2SO_4$. Appearance of reddish brown color at the junction between two layers, confirmed the presence of terpenoids [12].

**Test for anthraquinones:** 3 mL of 1% HCl was boiled with 150mg/ml sample extract and filtered. The filtrate was mixed with 5 mL of benzene, filtered followed by the addition of 2 mL of 10% ammonia solution. The mixture was shaken and the appearance of a pink, or red color in the ammoniacal phase indicated the presence of free hydroxyl anthraquinones [13].

**Test for coumarins:** 300 mg of CA-MeOH extract was added in test tube was covered with filter paper moistened with 2 N NaOH and placed in boiling water bath for few minutes. After removing the filter paper it was observed for yellow florescenceunder UV lamp, which indicated the presence of coumarins [14].

**Test for tannins:** 100 mg of CA-MeOH extract was boiled in 15mL of distilled water and filtered. Later few drops of 1% $FeCl_3$ were added, appearance of brownish green or a blue-black coloration revealed the presence of tannins [14].

**Quantitative analysis**

**Determination of total phenolic contents:** The total phenolic (TPC) of the plant extracts was determined according tothe Folin-Ciocalteu method [15], with slight modifications. Briefly 20 µL of sample extracts (1mg/ml) were mixed with 100 µL of Folin-Ciocalteu (10% diluted in water) reagent, the reaction mixture was pre incubated for 8 minutes and then 100 µL of sodium carbonate 20%, were added. The final solution was further incubated for 2 hrs at room temperature and the absorbance was measured in a spectrophotometer at a wavelength of 765 nm. Standard calibration curve of gallic acid was prepared at the concentration of 10, 20, 40 and 80 ug/ml. (Figure 1). TPC was expressed as gallic acid equivalents (GAE) in milligrams per g of extract.

**Determination of total flavonoid contents:** Total flavonoid contents were measured using spectrophotometric method [16], with some modifications. In detail , 20uL solution of each plant extracts (1mg/mL) in methanol were separately mixed with 10ul of 10% aluminium chloride, followed by addition of 10 uL of 1 M potassium acetate, and 100 uLof distilled water, and incubated for 30 minutes at room temprature. The absorbance of the reaction mixture was measured at 415 nm .Total flavonoid contents were calculated as rutin equivalent in milligrams per gram extract, from a calibration curve, which was prepared by making different concentration solutions10, 20, 40 and 80 ug/mL (Figure 2).

**Determination of antioxidative activity**

**DPPH radical scavenging assay:** The antioxidant activity of the extracts was assessed by the DPPH• radical scavenging ability using the method[17]. A volume of 100 uL of a solution of of 0.1mM DPPH• solution was mixed with 100 uL of the extract at various concentrations(10 to 500 µg/mL), the mixtures were left to stand in the absence of light for 30 minutes. After this time the absorbance was read at 515 nm in a spectrophotometer. Ascorbic acid was used as standard compound. The free radical scavenging activity was calculated as percentage of DPPH decolouration using the formula; Scavenging ability (%) = $[(A_0 - A_S)/ A_0] \times 100$, where $A_0$ is the absorbance of control (containing all reagents except the tested samples) and $A_S$ is the absorbance of tested sample.

**ABTS radical scavenging Assay:** Like DPPH assay, ABTS(2,2'– azinobis-(3-

ethyl-benzothiazoline- 6-sulphonic acid) assay is free radical scavenging assay. The test was performed by following the method [18],with little changes. ABTS free radical was generated by reacting ABTS solution (7mM) with Potassium per sulfate solution (2.45 mM). Stock solution was incubated under dark for about 14−16 h to produce stable free $ABTS^+$ radical. Before use, ABTS solution was diluted with methanol to give absorbance of 0.7± 0.4 at 735nm. 50µL sample extract at different concentrations (10 to 500 µg/mL) in MeOH was mixed with of 200 µL of $ABTS^+$ solution, kept of 5 minutes at room temperature under darkness. Ascorbic acid was used as standard solution, change in absorbance was measured at 735 nm and scavenging capacity was determined by equation, as described above in DPPH assay.

**RESULTS AND DISCUSSION**

**Phytochemical screening**: Crude methanol extract of root extract of *C. angustifolia* revealed the presence of alkaloids, saponins, anthraquinones, flavonoids and tannins (Table 1).The presence of these phytochemicals vary in different fractions. Flavonoids showed their existence in methanolic, ethyl acetate and butanol fractions, these polyphenol compounds are responsible to elicit free radical scavenging activities.

**Total phenolic content (TPC):** Phenolic compounds are most widely prevalent constituents of plants and their major sources in human diet are fruit, vegetables and various beverages. These polyphenol compounds includes, proanthocyanidins, flavonoids and derivatives of hydroxycinnamic acids, which are obtained from forest trees and from the industrial wood transformation residue [19].In order to evaluate the potential antioxidant capacity of the extracts from roots of *C. angustifolia*, it was important to determine the content of various polyphenols in methanol extract (CA-MeOH), Hexane fraction (CA-Hex ), Ethyl acetate fraction (CA-EA), Butanol fraction (CA-BuOH) and water fraction (CA-Water) . The total phenols, flavonoids, was determined by spectrophotometric methods and the results are presented in Table2**.**The amount of phenolic contents were calculated as their gallic acid equivalent (GAE), it was found in range from 122.56 ± 2.56 to 15.47 ± 5.44 mg GAE/g. CA-EA fraction showed highest phenolic contents 199.24 ± 1.25mg GAE/g, followed by CA-BuOH 166.25 ± 3.12 mg GAE/g. However CA-Water fraction expressed lowest phenolic contents 15.47 ± 5.44 mg GAE/g.

**Total flavonoid content (TFC)**: Total flavonoid contents varied in different extracts and ranged from 35.58 to 153.45

mg RU/g sample extracts (Table 2). The extract with highest TFC was CA-EA (153.45 ± 1.76 mg RU/g), indicating to be most active antioxidant fraction. TFC in all sample extracts were in following order: CA-EA > CA-MeOH> CA-BuOH> CA-Hex > CA-water.

**Antioxidation activity**

**DPPH assay:**DPPH is very stable free radical, it is unaffected by side reactions such as enzyme inhibition and metal ion chelation. A freshly prepared DPPH solution exhibits a deep purple colour with an absorption maxima at 517 nm. The purple color of DPPH free will become colorless upon reaction with antioxidant molecule and cause decrease in absorbance. The extent of antioxidation power is manifested from discoloration of DPPH free radical[20]. Concentration dependant response curve of DPPH scavenging activities was obtained with methanolic extract and its fractions. Antioxidation potential was confirmed by comparing their $IC_{50}$ values with standard ascorbic acid. CA-EA fraction was most ant oxidative with an $IC_{50}$ value of 50.63 ± 1.23 followed by CA-MeOH extract ($IC_{50}$= 132.90 ± 4.31) and CA-BuOH ($IC_{50}$= 171.68 ± 4.18) as shown in Table 3. CA-water fraction was the least active fraction due to highest $IC_{50}$value (317.33 ± 6.42).

**ABTS assay:** ABTS and DPPH assays are most commonly used invitro antioxidation methods. ABTS radical is generated by the reaction potassium sulphate with ABTS salt. ABTS radical solution is blue-green in color which becomes colorless due to donation of H atoms by antioxidant compound [21]. ABTS free radical scavenging capability of total extract and its fractions was observed in descending order as CA-EA > CA-MeOH> CA-BuOH> CA-Hex > CA-water fraction showing an $IC_{50}$ values as 55.04 ± 2.56, 135.44 ± 1.43, 173.60 ± 4.12, 207.66 ± 3.32, 346.10 ± 5.34 respectively. All these value were compared with that of standard ascorbic acid ($IC_{50}$= 32.60 ± 1.01). Lower$IC_{50}$ value of ethyl acetate fractionwas in good agreement with standard.

**Table 1: Phytochemical constituents of root extract of *Cassia angustifolia***

| Phytochemical test | CA-MeOH | CA-Hex | CA-EA | CA-BuOH | CA-Water |
|---|---|---|---|---|---|
| Alkaloids | + | − | + | − | − |
| Saponins | + | − | − | + | + |
| Terpenoids | − | + | + | + | − |
| Anthraquinones | + | + | + | − | + |
| Coumarins | − | + | + | − | − |
| Flavonoids | + | − | + | + | − |
| Tannins | + | − | + | + | − |

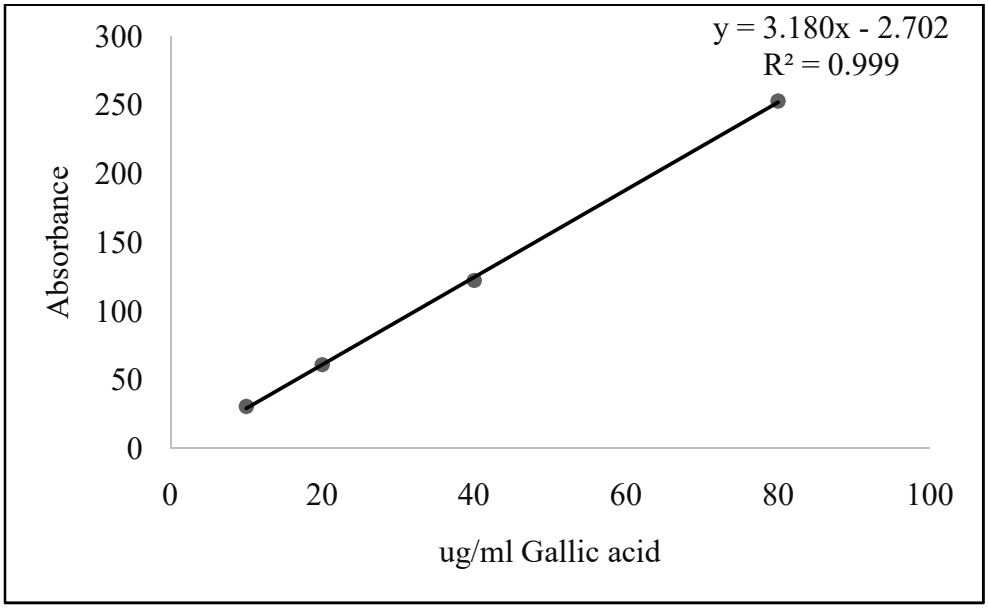


**Figure 1: Standard calibration curve for total phenolic contents.**

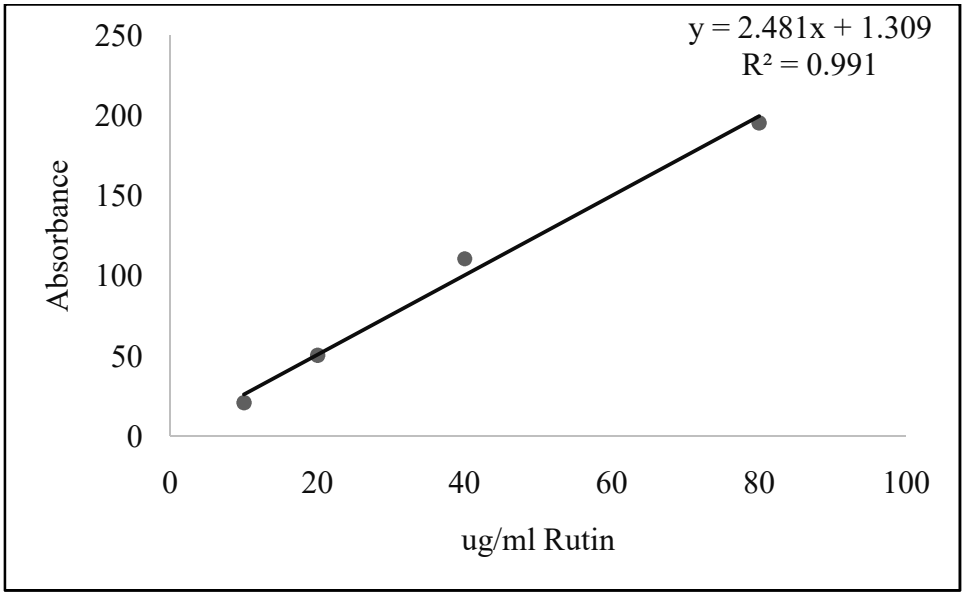


**Figure 2: Standard calibration curve for total flavonoid contents**

**Table 2: Total phenolic content (TPC) (mg GAE/ g dried extract) and total flavonoid content (TFC) (mg RU/mg dried extract) of root extract of *Cassia angustifolia***

| Sample | Total Phenols | Total flavonoids |
|---|---|---|
| | (mg GAE/g extract) | (mg RUE/g extract) |
| CA-MeOH | 122.56 ± 2.54 | 95.34 ± 3.43 |
| CA-Hex | 85.32 ± 3.53 | 40.78 ± 2.16 |
| CA-EA | 199.24 ± 1.25 | 153.45 ± 1.76 |
| CA-BuOH | 166.25 ± 3.12 | 74.66 ± 4.22 |
| CA-water | 15.47 ± 5.44 | 35.58 ± 4.97 |

**All the values are in ± standard deviation (n=3)**

**Table 3: Antioxidation activity of sample extract and its fractions with an $IC_{50}$ [ug/ml]**

| Sample | $IC_{50}$ [ug/mL] | |
|---|---|---|
| | DPPH | ABTS |
| CA-MeOH | 132.90 ± 4.31 | 135.44 ± 1.43 |
| CA-Hex | 206.65 ± 2.42 | 207.66 ± 3.32 |
| CA-EA | 50.63 ± 1.23 | 55.04 ± 2.56 |
| CA-BuOH | 171.68 ± 4.18 | 173.60 ± 4.12 |
| CA-water | 317.33 ± 6.42 | 346.10 ± 5.34 |
| Ascorbic acid | 20.50 ± 1.12 | 32.60 ± 1.01 |

**All the values are in ± standard deviation (n=3)**

## CONCLUSION

The need of natural antioxidants have been increasing due to their safety as compare to synthetic compounds, in this aspect plants have become of great interest to utilize them as antioxidants. The current study showed that methanolic roots extract and its fractions contain pharmacologically active secondary metabolites. The obtained results revealed that the methanolic extract and ethyl acetate fraction possessed the highest amount of flavonoids and phenolic compounds and exhibited potential antioxidant activities in both DPPH and ABTS invitro assay methods, when compared to other solvent fractions. In future this study can be extended to isolate these phytochemicals from root extract and their chemical structures could be elucidated through various spectroscopic methods.

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