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Research Article

QUALITATIVE AND QUANTITATIVE DETERMINATION OF SECONDARY METABOLITES AND ANTIOXIDANT POTENTIAL OF *CASSIA SOPHERA* LINN LEAF EXTRACTS

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ABSTRACT

Cassia sophera Linn (*C. sophera*) is a medicinally important plant belonging to the family of Caesalpiniaceae. The whole part of the plant is used as traditional folk medicine and is reported to possess analgesic, anticonvulsant, antioxidant, anti-inflammatory, hepatoprotective and antiasthmatic activity. The objective of this study was to screen the phytochemicals, estimate the content of alkaloids, phenolic and flavonoids compounds and determine the antioxidant capacity of the *C. sophera* leaves. Qualitative analysis of various phytochemical constituents and quantitative analysis of total alkaloids, phenol and flavonoids were determined by the well-known test protocol available in the literature. The ethanolic extract of leaves of *C. sophera* was studied for antioxidant activity on different *in vitro* models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay, Hydrogen peroxide (H_2O_2) and Nitric oxide (NO) radical scavenging method. Phytochemical analysis of ethanol and aqueous extract revealed the presence of alkaloids, glycosides, flavonoids, saponins, phenolics, proteins and amino acids, carbohydrate, diterpenes. The total alkaloids, phenolic and flavonoids content of *C. sophera* leaves of ethanolic extract were 0.439, 0.864, and 1.014/100mg respectively. Ascorbic acid used as standards was also evaluated for comparison. The extract showed dose dependent free radical scavenging property in the tested models. *C. sophera* leaves extract showed IC_{50} value 78.10µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=17.68µg/ml). For hydrogen peroxide method, IC_{50} value was found to be 73.17µg/ml, which compares favourable with ascorbic acid (IC_{50}=36.613µg/ml). In nitric oxide model, IC_{50} value was found to be 92.29µg/ml, which was comparable to that of ascorbic acid (IC_{50}=24.63µg/ml). The present study describes the phytochemical profile and antioxidant activity of *C. sophera* which can be further used for medicinal applications.

Keywords: *Cassia sophera* Linn, Qualitative, Quantitative phytochemical, Antioxidant activity.

1. INTRODUCTION

Plant products have been a part of phytomedicine since time immemorial. These can be derived from any part of the plant like leaves, flowers, bark roots, fruits, and seeds [1]. Herbal medicines have become more popular in the treatment of any diseases due to the popular belief that green medicine is safe, easily available and with fewer side effects. Many plants are cheaper and more accessible to most people especially in the developing countries than orthodox medicine, and there is a lower incidence of adverse effects after use. These reasons might account for their worldwide attention and use [2]. The medicinal properties of some plants have been documented by some researchers [3-5]. Medicinal plant constitutes the main source of new pharmaceuticals and healthcare products [6]. Extraction and characterization of several phytocompounds of these green factories have given birth to some high activity profile drugs [7]. Indeed, the market and public demand has been so great that there is a great risk that many medicinal plants today face either extinction or less of genetic diversity [8]. Knowledge of the chemical constituents of the plant is desirable because such information will be valuable for the synthesis of complex chemical substances. Reactive oxygen species (ROS) or oxygen free radicals can cause damage to cells and tissues during infections and various degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases, like Alzheimer's disease, mutations and cancer [9,10]. The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) have been restricted because of

serious concerns about their carcinogenic potential [10, 11]. Natural antioxidants, especially phenolics and flavonoids, are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods [12]. Numerous studies were carried out on plants with antioxidant properties [11-13]. However, there is still great interest in finding new antioxidants from natural sources. *C. sophera*, locally known as kasundi, is a medicinally important plant belonging to family caesalpiniaceae. It is one of the important medicinal plants in the tropical and subtropical region in Asia especially in India, Sri Lanka, Pakistan, Malaysia, Myanmar, Bangladesh and in most tropical countries [14, 15]. In ethno-botanical literature, the leaves are mentioned to be used for their anti-inflammatory, anti-rheumatic, and purgative property, as an expectorant for cough, cold, bronchitis, asthma, and in liver disorders [15]. Earlier studies have investigated on the pharmacological activities of the seeds of *C. sophera* including analgesic and anticonvulsant, antidiabetic, inhibition of lipid peroxidation, herbicidal, and fungicidal effects [16]. Phytochemical analysis of certain *Cassia* species led to the isolation of flavonoids, anthraquinones, proanthocyanidins and condensed tannins [17-19]. Despite the immense ethno-medicinal properties attributed to *C. sophera*, the reported phyto-pharmacological study on variety levels of this plant is relatively infrequent to the best of our knowledge. Therefore, the present study was aimed to evaluate and compare antioxidant activity of the ethanolic leaf extracts of *C. sophera*, by using classical in-vitro assays for the purpose of validating its ethno medicinal use.

2. MATERIAL AND METHOD

2.1. Plant material

Leaves of *C. sophera* were collected from local region in separate sterile bags from Bhopal, Madhya Pradesh in the month of October, 2019. Plant material (leaves part) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. The plant material was then shade dried without any contamination for about 3 to 4 weeks. Dried plant material was ground using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

2.2. Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India). All the chemicals and solvent used in this study were of analytical grade.

2.3. Defatting of plant material

Powdered leaves of *C. sophera* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using soxhlet apparatus. The extraction was continued till the defatting of the material had taken place.

2.4. Successive extraction with different solvents by soxhletion method

Forty gm of dried plant material was exhaustively extracted with different solvents (chloroform, ethyl acetate, ethanol and aqueous) using soxhletion method. The extract was evaporated above their boiling points. The dried crude concentrated extract was weighed to calculate the extractive yield then transferred to glass vials (6 ×2 cm) and stored in a refrigerator (4°C), till used for analysis [20].

2.5. Phytochemical screening

Phytochemical screening to detect the presence of bioactive agents was performed by standard procedures [21, 22]. After the addition of specific reagents to the solution, the tests were detected by visual observation of color change or by precipitate formation.

2.6. Total phenol determination

Total phenolic content was determined using the method of Olufunmiso *et al* [23]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 10min for colour development. The absorbance was measured at 765 nm using a UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

2.7. Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [23]. 1ml of 2% $AlCl_3$

solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

2.8. Total alkaloids determination

The plant extract (1mg) was dissolved in methanol, 1ml of 2 N HCl ws added and filtered [24]. The solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120 µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

2.9. Antioxidant activity

2.9.1. DPPH radical scavenging assay

DPPH scavenging activity was measured by modified method of Olufunmiso *et al.,* 2011 [23]. DPPH scavenging activity was measured by the spectro-photometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentrations (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume was made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentrations were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally, the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation:

% inhibition = {(absorbance of control - absorbance of sample)/absorbance of control}× 100%.

Though the activity is expressed as 50% inhibitory concentration (IC_{50}), IC_{50} was calculated based on the percentage of DPPH radicals scavenged. The lower the IC_{50} value, the higher is the antioxidant activity.

2.9.2. Nitric oxide ($NO^{\bullet}$) radical scavenging assay

The determination of $NO^{\bullet}$ radical scavenging ability of the extracts is based on the inhibition of $NO^{\bullet}$ radical generated from sodium nitroprusside in phosphate buffer saline solution by Griess reagent (1% sulfanilamide, 2% orthophosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride). Scavengers of nitric oxide act against oxygen, prompting to lessened production of nitrite ions which can be monitored at 546 nm [25]. Briefly, sodium nitroprusside (0.6 ml, 5 mM) solution was mixed with and without varying the concentration of the extracts or Ascorbic acid (2 ml, 10-200µg/ml) and incubated at 25±2°C for 5 h. Incubated solution (2 ml) was mixed with equal volume of Griess reagent and absorbance of the purple colored azo dye chromophore was measured at λmax 546 nm using UV-Vis spectrophotometer. The NO^{o} radical scavenging ability was calculated using following formula:

Scavenging activity (%)= $\{(Abs_{control}-Abs_{sample})/Abs_{control})\}$ x 100

2.9.3. Free radical scavenging activity (FRSA) using hydrogen peroxide

Scavenging activity of hydrogen peroxide (H_2O_2) by the plant extract was determined by the method of Ruch *et al.,* (1989) [26]. Ethanolic extract (4 ml) prepared in distilled water at various concentrations was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm. Ascorbic acid was used as a positive control compound. The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples using following equation.

$S\% = \{(A_{control} - A_{sample})/A_{control}\} \times 100$

Where $A_{control}$ = absorbance of the blank control (containing all reagents except the extract solution), A_{sample} = absorbance of the test sample.

3. RESULTS AND DISCUSSION

The crude extracts so obtained after each of the successive soxhletion extraction process were concentrated on water bath by evaporating the solvents completely to obtain the actual yield of extraction. The

yield of extracts obtained from the leaves of *C. sophera* using chloroform, ethyl acetate, ethanol and water as solvents are depicted in the Table 1.

Table 1: Results of percentage yield of leaves extracts

Solvents	Percentage Yield
Chloroform	2.3
Ethyl acetate	6.8
Ethanol	9.5
Aqueous	11.6

The results of qualitative phytochemical analysis of the crude powder leaves of *C. sophera* are shown in Table 2. Ethanolic and aqueous extracts of leaves sample of *C. sophera* showed the presence of alkaloids, glycosides, flavonoids, saponins, phenolics, proteins and amino acids, carbohydrate, diterpenes but in chloroform extracts, all the phytoconstituents were absent and flavonoids was present in ethyl acetate extract. Total phenolic compounds (TPC) was expressed as mg/ 100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: $Y = 0.011X + 0.011$, $R^2 = 0.998$, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: $Y=0.032X + 0.018$, $R^2=0.998$, where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: $Y=0.007X+ 0.024$, $R^2=0.995$, where X is the Atropine equivalent (AE) and Y is the absorbance. The total phenolic, flavonoids and alkaloid estimation of ethanolic extracts of leaves of *C. sophera* showed the content values of 0.864, 1.014 and 0.439 respectively (Table 3). DPPH radical scavenging assay measured hydrogen donating nature of extracts [27]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *C. sophera* ethanolic leaves extract was found to be 79.10µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed (Table 4 & Fig. 1). Extracts showed $NO^{\bullet}$ scavenging effects by competing with oxygen to react with $NO^{\bullet}$ directly, hence inhibited the nitrite ion formation [28]. *C. sophera* ethanolic leaves extract showed nitric oxide ($NO^{\bullet}$) radical scavenging activity with IC_{50} value of 92.29µg/ml as compared to that of ascorbic acid (IC_{50} 24.63µg/ml) (Table 5 & Fig. 2).

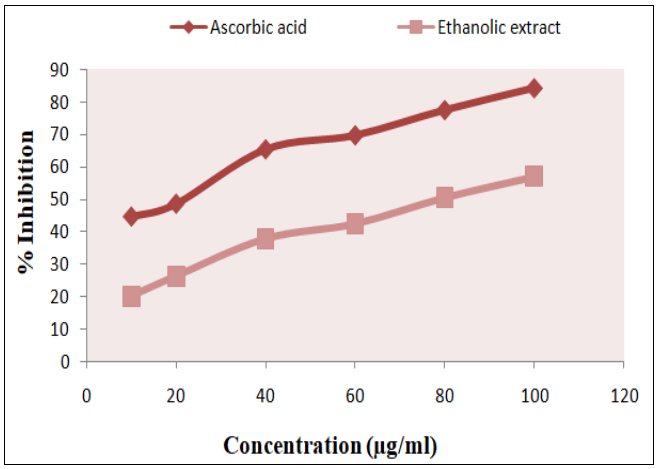

Fig. 1: % Inhibition of ascorbic acid and ethanolic extract of *C. sophera* using DPPH

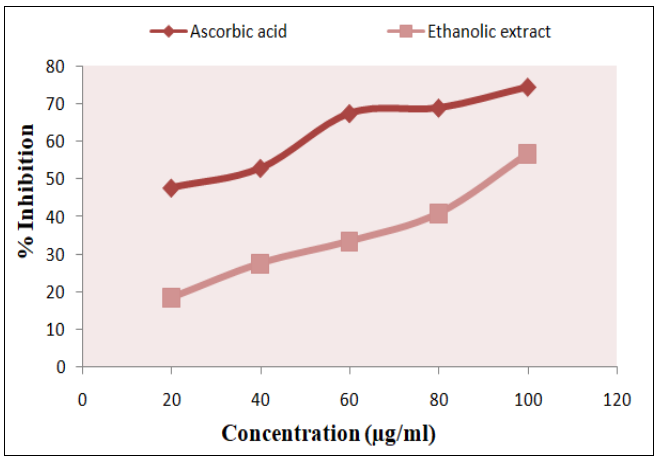

Fig. 2: % Inhibition of ascorbic acid and ethanolic extract of *C. sophera* using NO method

Hydrogen peroxide is generated *in vivo* by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains [29]. There is increasing evidence that, hydrogen peroxide, either directly or indirectly via its reduction product, $OH^{\bullet}$, can act as a messenger molecule in the synthesis and activation of several inflammatory mediators [30]. When a scavenger is incubated with H_2O_2 using a peroxidase assay system, the loss of H_2O_2 can be measured. Table 6 & fig. 3 show the scavenging ability of *C. sophera* ethanolic leaves extract and ascorbic acid on hydrogen peroxide at different concentrations. Extracts was capable of scavenging hydrogen peroxide in an amount dependent manner at all the tested concentrations. Hydrogen peroxide itself is a rather weak oxidant and most organic compounds (except for some sulfur containing

molecules) are virtually inert to attack by it at ordinary environmental or cellular concentrations and temperatures. In the presence of reduced transition metal ions, however, hydrogen peroxide is converted to the much more reactive oxidant, hydroxyl radical in the cells by Fenton reaction. Besides this, studies have shown that other transition metals such as copper (I), cobalt (II) and nickel (II) also take part in the process [29]. Thus, the removing is very important for antioxidant defense in cell or food systems.

Table 2: Result of phytochemical screening of extracts of *C. sophera*

Constituents	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
Alkaloids				
A) Wagner's Test:	-Ve	-Ve	-Ve	-Ve
B) Hager's Test:	-Ve	-Ve	+Ve	+Ve
Glycosides				
A) Legal's Test:	-Ve	-Ve	-Ve	+Ve
Flavonoids				
A) Lead acetate Test:	-Ve	+Ve	+Ve	+Ve
B) Alkaline Reagent Test:	-Ve	-Ve	-Ve	+Ve
Saponins				
A) Froth Test:	-Ve	-Ve	+Ve	+Ve
Phenolics				
A) Ferric Chloride Test:	-Ve	-Ve	+Ve	-Ve
Proteins and Amino Acids				
A) Xanthoproteic Test:	-Ve	-Ve	+Ve	+Ve
Carbohydrate				
A) Fehling's Test:	-Ve	-Ve	+Ve	+Ve
Diterpenes				
A) Copper acetate Test:	+Ve	-Ve	-Ve	+Ve

Table 3: Estimation of total phenolic, flavonoids and alkaloid content of leaves extract of *C. sophera*

S. No	Extract	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	Total alkaloid content (mg/ 100 mg of dried extract)
1	Ethyl acetate	-	0.769	-
2	Ethanol	0.864	1.014	0.439
3	Aqueous	-	0.831	0.374

Table 4: % Inhibition of ascorbic acid and ethanolic extract of *C. sophera* using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	44.65	20.06
2	20	48.62	26.25
3	40	65.34	37.85
4	60	69.65	42.48
5	80	77.41	50.57
6	100	84.13	57.03
	IC_{50}	17.68	79.10

Table 5: % Inhibition of ascorbic acid and ethanolic extract of *C. sophera* using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	47.70	18.47
2	40	52.92	27.64
3	60	67.43	33.51
4	80	68.89	40.95
5	100	74.42	56.84
	IC_{50}	24.63	92.29

Table 6: % Inhibition of ascorbic acid and ethanolic extract of *C. sophera* using H_2O_2 method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	42.87	25.87
2	40	52.19	38.57
3	60	60.41	45.96
4	80	65.78	52.47
5	100	68.75	60.18
	IC_{50}	36.61	73.17

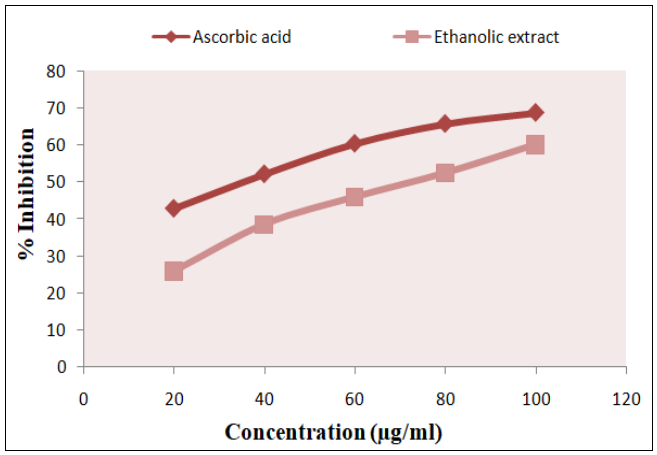

Fig. 3: % Inhibition of ascorbic acid and ethanolic extract of *C. sophera* using H_2O_2 method

4. CONCLUSION

Due to the presence of a good number of flavonoid, alkaloid and phenolics content and appreciable quantities of secondary metabolites in leaves parts of the plant studied here, the plant can be seen as a potential source of useful drugs. The presence of phyto-constituents in a considerable amount might serve to recognize the potential pharmacological importance of this plant in disease control. The medicinal value of plants lies in some chemical substances that have a definite physiological action on human body. It also justifies the folklore medicinal uses and claims about the therapeutic values of this plant as curative agent. We therefore suggest further the isolation, purification, and characterization of the bioactive compounds from leaf, stem, flower, and seed of *C. sophera* with a view to obtain useful chemotherapeutic agents.

Conflict of interest

None declared

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