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IN VITRO ANTIOXIDANT EFFECTS OF HYDROALCOHOLIC EXTRACT OF CASSIA TORA AERIAL PART USING DIFFERENT MODELS

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ABSTRACT

Background: Free radicals, harmful by-products of a cell's natural metabolism, are responsible for various health problems. The search for plant-based supplements or medicines is always in high demand, as is the antioxidant activity that contributes to the therapeutic efficacy of plants. **Aim:** In the present study, the hydroalcoholic extracts from the aerial parts of *Cassia tora* were used for in vitro analysis of their antioxidant activity. **Methods:** Six separate assay methods were used to evaluate the antioxidant activity, i.e., against hydroxyl radical, DPPH, superoxide anions, nitric oxide, and also total flavonoid and phenolic content, were investigated. This was done by standardizing hydroalcoholic extract (70/30 ethanol to water) of Cassia tora and ascorbic acid. **Results:** Percentage scavenging activity and IC_{50} value were measured for extract prepared at various concentrations. The results IC_{50} values were 25.54 µg/ml, 45.04 µg/ml, 36.56 µg/ml, and 97.61 µg/ml for DPPH, superoxide radicals, hydroxyl radicals, and nitric oxide, respectively. Subsequently, the total phenolic and flavonoid content in the extract obtained was 1.927±0.73 mg GAE/gm and 1.018±0.29 mg QE/gm, respectively. **Conclusion:** The hydroalcoholic extract of *Cassia tora* contains more phytoconstituents. This suggests it has a wide range of medicinal antioxidant properties that make it helpful in treating many diseases. With the increasing demand for safer herbal treatments, scientific efforts in this field are making significant contributions and advances and supporting innovation.

INTRODUCTION

It is well known that antioxidants are nothing but molecules that limit the oxidative process that leads to the formation of free radicals (with a single unpaired electron), which trigger a chain of reactions that damage normal cells [1, 2]. The subsequent series of cellular damage triggered by free radicals is called oxidative stress, which is associated with the cause of numerous diseases such as heart, CNS, bronchitis, arthritis, cancer, inflammatory diseases, etc. These processes cause further complications by releasing more free ions (Fe or Cu), synthesizing enzymes that produce molecules with unpaired electrons, disrupting redox processes, etc. Under normal

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conditions, the body has several endogenous enzymes that act as antioxidants, namely catalase, glutathione peroxidase, superoxide dismutase, etc., which can neutralize free radicals and deactivate the enzymes responsibly, thus preserving ideal cellular functions[3]. However, these visceral antioxidants may prove ineffective under increased oxidative stress, suggesting the need for external antioxidants. The fear of deadly diseases that have no known causes has increased the risks to humanity worldwide, including the risks from the side effects of conventional, synthetically produced drugs such as antioxidants (Butylated hydroxyl-anisole/ toluene). This has increased interest in using natural, ingenious medicinal and nutritional plants as therapeutic alternatives, including antioxidants [4, 5]. The medicinal potential of a plant is well documented due to the presence of secondary metabolites such as phenolics, flavonoids, alkaloids, terpenoids, vitamins, tannins, etc. Many plants have been included in this category, with most having antioxidant compounds, including polyphenolic compounds, namely phenolics and flavonoids, which are grouped differently based on their structural characteristics. They are all identified as naturally occurring in plants and can also scavenge free radicals or active oxygen. These polyphenols come from plants with at least one hydroxyl group on their aromatic ring. This hydroxyl group acts as a simple electron donor, directly allowing them to develop their antioxidant effect [6, 7]. Since ancient times, people have used various herbal products in both raw and refined forms in food, cosmetics, and treatments to make them safer and more affordable. As a result, the WHO and other regulatory bodies have established standardized protocols for using plants or plant derivatives in medicine [8, 9]. To avoid unwanted side effects, the concept of multi-targeting with one drug has been replaced by a single drug with specific targeting. Besides the unpleasant effects of conventional allopathic medicines, most of the world's population trusts herbal medicines as the first choice therapy, as mentioned in the holy scriptures [10, 11]. *Cassia tora* is an uninhabited species of plant that is well known and a component in South Asian medicine, including the Indian and Central American folk medicine systems, for its several properties [12]. According to the Ayurveda recommendations, cassia and its parts are traditionally prescribed in conditions related to CVS, lungs, intestine, leprosy, malaria, etc. The seeds have also been found to lower BP, physical weakness, cholesterol, etc. These effects of *Cassia tora* have been attributed to its elements with responses like antagonistic effects for oxidative stress, hepatic toxicity, inflammation, plasmodial cycle, etc [13, 14]. The antioxidant property is ascribed to phytochemicals, i.e., polyphenols, emodin, chrysophanols, anthraquinones, flavonoids, apigenin, across, later in, etc. *Cassia tora* extracts have also been reported as potent antioxidants due to their polyphenols in optics like xerophthalmia and cataracts [15]. Additionally, *Cassia tora* seeds in Korea are roasted and consumed as coffee, which is helpful for diabetics [16, 17]. Thus, several ethnomedicinal uses of Cassia tora suggest that the plant can protect cells and, thus, optimal functioning from damage due to oxidative stresses. These effects, i.e., antioxidant, are related to its component's ability to act as scavengers from radicals, donors of hydrogen, electrons, decomposers of peroxides, peroxy-nitrites, singlet charged oxygen, etc [18]. To date, antioxidant models for Cassia tora extract have been described, but these are very few in number and lack in one or the other aspect. It has been observed that antioxidants possess various medicinal properties for curing different types of diseases. Once the antioxidant activity of *Cassia tora* is confirmed, new opportunities will open for researchers to study the therapeutic efficacy based on antioxidant activity. Therefore, the study's objective was to determine the antioxidant activity of the hydroalcoholic extract from the aerial parts of *Cassia tora* using in vitro models. Predictions of antioxidant activity should not be derived solely from the results of a single antioxidant test model. Different in vitro test models are performed to evaluate the antioxidant activity of the extract in question. It should also be noted that the antioxidant test models vary in different aspects. Therefore, to better understand the antioxidant activity of the extract, it must be tested with different models. This will assist researchers by supplementing with base work information and enhance the literature pool about medicinal plants' properties for future advancements.

MATERIALS AND METHODS

Cassia tora Collection

For the present work, the aerial parts of *Cassia tora* were gathered. The plant parts were compiled from Vindhya Herbals MFP-PARC at Bhopal, MP, India. After cleaning, these parts were shade-dried and coarsely powdered for further study. Loba Chemie, Merck chemicals, and Sigma-Aldrich chemicals and reagents were used for this study.

Extract preparation

Maceration methods were used to extract the phytoconstituents from the coarsely pulverized *Cassia tora*. The menstruum was placed in the container with the plant powder until it completely

covered the drug. The container was closed and kept at room temperature for seven days, stirring the contents regularly. In preparing the extract, cleaned, shade dried, coarsely powdered 250 gm of the drug was first defatted using petroleum ether. This is because it has been reported that the defatting process adds to the antioxidant property. Further, hydroalcoholic (ethanol: water in 70:30 ratio) solvent was used to extract the desired components using the maceration method. The hydroalcoholic solvent was chosen because the flavonoids and polyphenol components are more soluble in this solvent than in non-polar and semi-polar solvents. The hydroalcoholic solvent ensures maximum flavonoids and polyphenol components extraction from the *Cassia tora*. The resulting hydro-alcoholic extract was then used to determine antioxidant activity in vitro.

Antioxidant activity analysis In-vitro of resulting extract of *Cassia tora*

Inhibitory effect over DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical

Guchu et al. described a method for estimating the DPPH scavenger effect that was slightly modified for this study. For this assay, samples of the extract were prepared at different strengths, i.e., 50, 100, 150, 200, and 250 µg/ml. Methanol was used as a solvent for the preparation of all extract samples. A standard ascorbic acid solution with a similar concentration to the test solution was also prepared. In a clean test tube, a 1-ml test sample was mixed in 0.5 ml, 0.3 mM methanolic DPPH solution, and stirred until the solutions were mixed entirely. After preparation, solutions were kept in a dark room at normal room temperature for about 15 min. The solution as control consisted of a combination of 1 ml of methanol and 2.5 ml of DPPH solution. The sample absorbance was measured at 517 nm employing a spectrophotometer, and the following formula was considered to compute the percentage of inhibitory activity [19, 20]:

$$\%Radical\ inhibiting\ activity = \frac{C - S}{C} \times 100$$

C - Amount of absorbance by the control solution

S - Amount of absorbance by the extract/standard solution

The IC_{50} value of the extracted sample and the standard solution was measured. Three replicates of each experiment were performed, and the mean values of the results were calculated.

Inhibitory effect over Hydroxyl Radical

As Kotha et al. described, the hydroxyl radical's inhibitory activity was calculated using the method with required modifications. The test samples were generated by pouring 1 ml of each of the six strengths of the extract (i.e., 50, 100, 150, 200, and 250 µg/ml) into a mixture that contained 0.1 ml EDTA (1 mM), 0.36-ml deoxyribose, 0.01 ml (10 mM) $FeCl_3$ (10 mM), 0.1 ml H_2O_2 (10 mM), and 0.33-ml phosphate buffer (50 mM, pH 7.9). A standard ascorbic acid solution with a concentration similar to the test solution was also prepared. Later, sample mixtures were allowed to stand for 1 hour at 37 °C. Finally, 1 ml of samples incubated was treated with 1 ml of each 10% TCA and 0.5% TBA. The absorption of the prepared mixture was then measured at 532 nm by employing a spectrophotometer, and the equation below was considered to compute the percentage of inhibitory activity [21, 22]:

$$\%Hyroxyl\ radical\ inhibiting\ activity = \frac{C - S}{C} \times 100$$

C - Amount of absorbance by the control solution

S - Amount of absorbance by the extract/standard solution

The percentage of hydroxyl radical scavenger of the extract or standard represents the percentage inhibition of deoxyribose. The IC_{50} value of the extracted sample and the standard solution was measured. Three replicates were performed for each experiment, and the mean values of the results were calculated.

Inhibitory effect over Superoxide anion radical

A modified approach published by Sivagamasundari et al. determined activity inhibitory on superoxide anions. Test samples were prepared by mixing 0.5 ml NADH (0.936 mM), 0.5 ml of nitroblue tetrazolium (NBT) (0.3 mM), 1.0 ml extract at different strengths (i.e., 50, 100, 150, 200, and 250 µg/ml), and 0.5 ml Tris-HCl buffer (16 mM, pH 8.0). Then, 0.5 ml of phenazine methosulfate (PMS) (0.12 mM) was added to the above mixture for reaction. Further, it was kept at a standstill at 25°C for 5 mins to get the final incubated solutions for evaluation. The method was then repeated for an ascorbic acid standard solution with a concentration similar to the test solution. When working with a spectrophotometer, it was possible to assess the absorption of the sample at 560 nm, and the following formula was considered to compute the percentage of inhibitory activity [22]:

$$\%Superoxide\ radical\ inhibiting\ activity = \frac{C - S}{C} \times 100$$

C - Amount of absorbance by the control solution

S - Amount of absorbance by the extract/standard solution

The IC_{50} value of the extracted sample and the standard solution was measured. Three replicates of each experiment were performed, and the mean values of the results were calculated.

Inhibitory effects over Nitric oxide

The inhibitory effect of nitric oxide was estimated by a modified technique mentioned by Sashikumar et al. 3 ml of test solution was prepared by mixing 0.5 ml phosphate buffer saline having pH of 7.4, 2 ml sodium nitroprusside 9SNP0 (10 mM), and 0.5 ml of different strengths of the extract (i.e. 50, 100, 150, 200, and 250 µg/ml), and further incubated or kept standstill for 150 min at 25 °C. Further, 0.5 ml of this solution was pipetted off and replaced with 1 ml sulfanilic acid reagent. The resulting solution was left undisturbed for 5 minutes to complete the diazotization process. 1 ml of naphthyl ethylenediamine dihydrochloride was mixed with the resulting solution, and this was then incubated for 30 minutes at 25 °C. The same process was followed to prepare a standard ascorbic acid solution with a concentration similar to the test solution's. When working with a spectrophotometer, it was possible to assess the absorption of the sample at 540 nm, and the following formula was considered to compute the percentage of inhibitory activity [23]:

$$\%NO\ inhibiting\ activity = \frac{C - S}{C} \times 100$$

C - Amount of absorbance by the control solution

S - Amount of absorbance by the extract/standard solution

The IC_{50} value of the extracted sample and the standard solution was measured. Three replicates of each experiment were performed, and the mean values of the results were calculated.

Total phenolic content (TPC) determination in extract

In this method, 2 ml extract was mixed thoroughly with 1 ml of Folin Ciocalteu reagent (1:10 v/v), and the resulting solution was held for 7 min. Folin Ciocalteu reagent forms a blue complex when combined with phenolic chemicals in the extract. To the reaction mixture, 1 ml sodium carbonate solution (7.5 g/ l) was poured and kept for 2 hours in a darkened room. At 760 nm, the absorption values were measured using spectrophoto-meterically. TPC was measured using a standard gallic acid solution curve of 10-50 µg/ ml, and outcomes were expressed as gallic acid equivalents (mg GAE/ gm extract). Three replicates of each experiment were performed, and the mean values of the results were calculated [24].

Total flavonoid content (TFC) determination in extract

Add 0.5 ml of $AlCl_3$ solution (2%) to the 0.5 ml of extract, and further allow it to be kept for 1 hour at 25 °C. Aluminum tri-chloride forms a colorful complex that can be detected Spectrophotometrically in the presence of a base. Afterward, using a spectrophotometer, the absorption at 420 nm of the samples was determined. TFC was then measured through a standard quercetin curve (10 - 50 µg/ ml), and outcomes were represented as quercetin equivalents (mg QE/ gm extract). Three replicates of each experiment were performed, and the mean values of the results were calculated [23].

Statistical analysis

The TFC and TPC curve data were evaluated using Microsoft Excel 19. The data was examined three times and presented as the means ± standard deviation (SD).

RESULTS AND DISCUSSIONS

It is well known that in vitro studies of antioxidant activity cannot rely on the results of a single assay model. Keeping this in mind, 4 different radical scavenging methods, namely superoxide, DPPH, nitric oxide, and hydroxyl radical scavenging methods, were used to confirm the antioxidant activity of the hydroalcoholic extract from *Cassia tora*. These radical scavenging assays and the spectrophotometric methods confirmed TPC and TFC in the polyphenolic extract of the Cassia tora medicinal plant, suggesting its use as a potent antioxidant in clinical recommendations.

Inhibitory effect of the extract on DPPH radical

Based on the assumption that the hydroalcoholic extract of *Cassia tora* can donate hydrogen atoms, a free radical DPPH assay was conducted to prove this. The outcomes of the study are charted in Figure 1, showing hydroalcoholic extract free radical scavenging at 5 different strengths of extract, i.e., 50, 100, 150, 200, 250 µg/ ml, keeping ascorbic acid (in the same concentration) as standard. Both test (hydroalcoholic extract) and standard (ascorbic acid) extracts exhibited IC_{50} values of 25.54µg/ ml and 12.45µg/ ml, respectively. These extract IC_{50} values were considerable compared to the ascorbic acid, and an antiradical effect was found proportional to the concentration of extract. DPPH is the most commonly used in vitro assay method for properties because it costs less, is shorter, and is affordable. Several plant extractions have been assayed for their antiradical activity using DPPH. In a study by Siddartha et al. on *Ficus religiosa*, they reported antioxidant activity of 0.550 at 517nm absorbance, with the percent antioxidants being calculated as 43.415% [25]. In the DPPH assay, a radical scavenger compound reduces DPPH to DPPH-H (a hydrazine form), lowering its absorbance and decolorizing it from a purple to

yellow hue. In another study on the antioxidant activity of *Vernonia amygdalina* by Huseen et al., where they used different leaf extracts (in H_2O, MeOH, and EtOH), IC_{50} value obtained through DPPH assay was 111.4, 94.92, and 94.83 µg/ml [26]. In another study, Luhata et al. assayed ethyl acetate extract of Odontonema striatum leaf using the DPPH method for antioxidant action of 4 different α-pyrones and got IC_{50} values in the range from 0.24±0.0002 µg/mL to 55.7±0.027 µg/mL against the standard ascorbic acid with IC_{50} value 1.73×10-3±0.3µg/ ml [27]. The discussions and results collected in the data presented show that the extract can solubilize hydrogen ions against the DPPH free radicals. This proves that the *Cassia tora* extract can potentially reduce DPPH radicals by converting them into equivalent hydrazine.

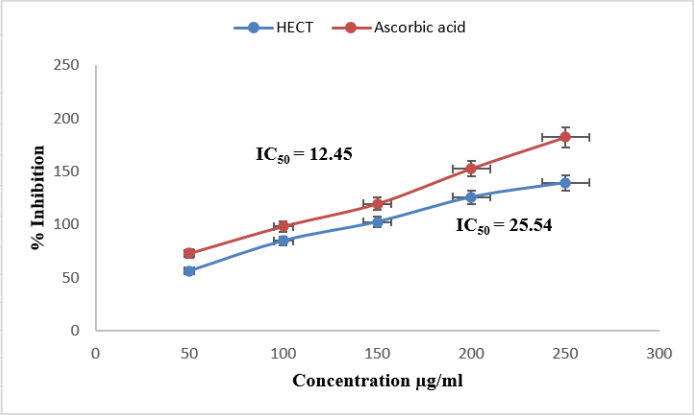

Figure 1: Graphical representation of scavenging abilities of *Cassia tora* on DPPH radicals; HECT IC_{50} values - 25.54µg/ ml and Ascorbic acid IC_{50} - 12.45µg/ ml

Inhibitory effects of the extract on the Hydroxyl Radical

It was proven that the hydroalcoholic extract of Cassia tora could be attributed to antioxidant activity proportional to the phyto-compound concentration in the extract solution shown in Figure 2, using ascorbic acid as standard, to destroy and subdue hydroxyl radicals' formation successfully. IC_{50} values of 36.56µg/ml and 97.69µg/ml for hydroalcoholic extract and ascorbic acid, respectively, were established. This assay for antioxidant activity was performed to establish Cassia tora polyphenols' involvement in •HO free radical reduction. Reduced dioxygen's highly reactive forms are Hydroxyl radicals. They are known to be involved directly in DNA irreversible damage by interfering with thiol groups, denaturating a number of physiological enzymes resulting in fibrinolysis, carcinogenesis, etc [28]. Also, studies have shown that •HO radicals alter membrane unsaturated fatty acids, resulting in oxidative stress-related complications demanding the need for effective antioxidants [29].

Several different studies were carried out to demonstrate the antioxidant effects through the scavenging effect of hydroxyl radicals. Carolyn et al. studied the antioxidant nature of C. edulis and *P. capensis* extracts using the Fenton reaction and obtained IC_{50} values between 101 and 150 µg/ml. The protective effect was based on a reduction in pink chromogen [Carolyn Wanjira]. Similarly, Daniele et al. investigated the antioxidant effects of various plant extracts, including ginger, blueberry juices, and green tea infusion, using the Fenton reaction. To measure the antioxidant potential, they examined the EC_{50} values, i.e., the extract concentrations that reduce the amount of DMPO-OH adducts by half. They discovered that these two values are inversely proportional to each other. [30]. In another antioxidant study by Hussen et al. on leaf extract of Vernonia amygdalina, IC50 values of 141.6, 156, and 180.6 µg/ml in H2O2 assay using H2O, MeOH, and EtOH extracts [26]. The results of the present study show that the extract of *Cassia tora* strongly limits the formation of hydroxyl radicals and thus demonstrates its significant antioxidant potential.

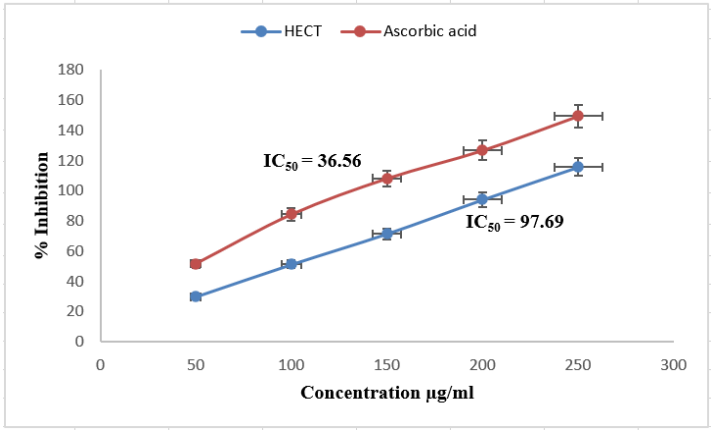

Figure 2: Graphical representation of scavenging abilities of *Cassia tora* on hydroxyl radicals; HECT IC_{50} values – 97.69µg/ ml and Ascorbic acid IC_{50} – 36.56µg/ ml

Inhibitory effect of the extract on Superoxide anion radical

Superoxide anions (i.e., $O2^{\bullet(-)}$), highly reactive oxygen atoms, have been vividly shown to cause severe oxidative stress damage affecting various lipids, proteins, etc., and disrupting cell structure. Reports show these results from NBT's oxidative reduction during the PMS/NADH coupling process. Therefore, a superoxide anions antiradical assay can be very helpful in establishing antioxidant activity. In the present study, the

hydroalcoholic extract of Cassia tora showed IC50 values of 45.04 µg/ml against the ascorbic acid standard with IC50 values of 16.13 µg/ml for superoxide radical reduction (Figure 3). Likewise, Hieu et al. proved the antioxidant effect of anthraquinone derivative Rubiadin (RBD) from Rubia cordifolia in an aqueous solution. They reported the component showed noteworthy scavenging activity for superoxide anion radical (k = 4.93 × 10^8 M^{-1} s^{-1}). In addition, antioxidant activity against $CCl_3O^{\bullet}$, $CCl_3OO^{\bullet}$, NO_2, $SO_4^{\bullet-}$, and $N_3^{\bullet}$ radicals as well [31]. In another study by Kour et al. with a methanolic extract from the bark of C. fistula, a maximum antioxidant effect was observed with an IC_{50} value of 29.41 against the superoxide anion assay. The effect was shown to be proportional to extract concentration [32]. Following the above-mentioned studies, our findings also indicate considerable antagonistic superoxide anion activity of Cassia tora extract compared to the ascorbic acid. Also, the extract's $O_2^{\bullet(-)}$ reducing effect was in a concentration-dependent manner.

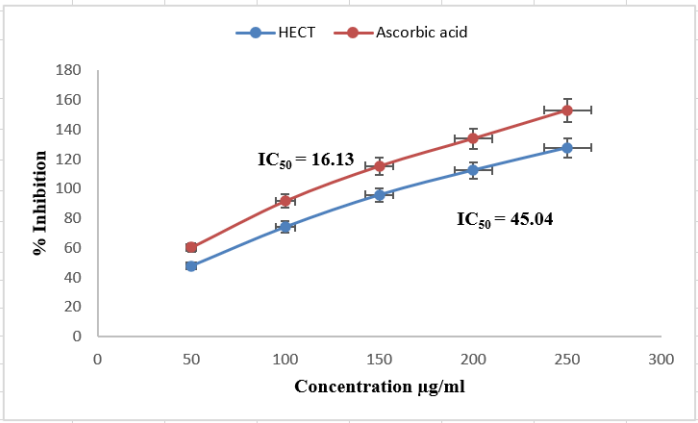

Figure 3: Graphical representation of scavenging abilities of *Cassia tora* on superoxide radicals; HECT IC_{50} values – 45.04µg/ ml and Ascorbic acid IC_{50} – 16.13µg/ ml

Inhibitory effect of the extract on Nitric oxide free radical

Nitric oxide (NO) is the most lethal and least charged of the free radicals researched to date. The lethal effect of NO is enhanced by the presence of oxygen/superoxides, with which it reacts to produce peroxynitrite. These trigger and develop cell damage and inflammatory reactions. In NO assay, the antioxidant compound in plant extract competitively inhibits free radical formation by a priory binding with superoxides/oxygen molecules. In the present study, the antioxidant activity against NO radicals resulted in an IC_{50} value of 97.61µg/ ml for hydroalcoholic extract and 48.76µg/ ml for standard ascorbic acid (Figure 4). These findings are in agreement with the study by Mueed et al., in which they investigated 7 different plant species, among which Mentha arvensis exhibited the highest antiradical effect, i.e., 81.71±1.1% with an IC50 value of 132.8±3 [33]. A study by Kour et al. on *Cassia fistula* bark extract in methanol also showed no antiradical effect with an IC_{50} value of 13.38 µg/ml, which underlines a positive relationship with the polyphenol concentration of the extract [32]. The findings presented in this paper, along with the hydroalcoholic extract of Cassia tora, also inhibited NO radicals in a dose proportional manner. This demonstrates the robust antioxidant capacity of *Cassia tora*, indicating its usefulness in several clinical situations pathologically.

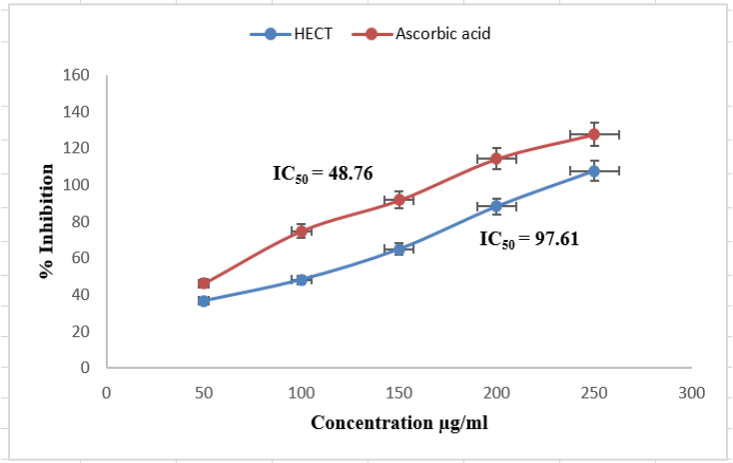

Figure 4: Graphical representation of scavenging abilities of *Cassia tora* on nitric oxide radical; HECT IC_{50} values – 97.61µg/ ml and Ascorbic acid IC_{50} – 48.76µg/ ml

Total flavonoid and phenolic content of extract

The calibration curve of gallic acid and quercetin is represented in Figure 5 and Figure 6, respectively. The results quantitatively indicate that the hydroalcoholic extract of *Cassia tora* contains 1.927±0.73 mg GAE/gm of phenolic compounds and 1.018±0.29 mg QE/gm of flavonoid compounds. This quantitative estimation of the plant content in extract shows its ability for antioxidant effects. Similar results were obtained in an earlier study by Kumar et al. in which the ethanolic extract of *Cassia tora* leaves had a flavonoid content of 106.8 ± 2.8mg/ g dry weight QE. Phenols and flavonoids generally occur together in medicinal plants [34]. Among other bioactivities, they exhibit strong antioxidant properties due to their redox potential, which donates hydrogen atoms and acts as a reducing agent. Thus, we also achieved Cassia tora extract with unexpectedly high concentrations of phenolic and flavonoid components in the present study.

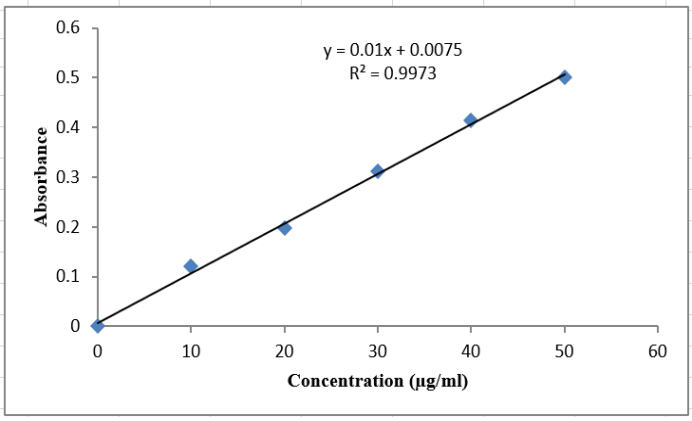

Figure 5: Gallic acid calibration curve employed for quantifying phenolic content

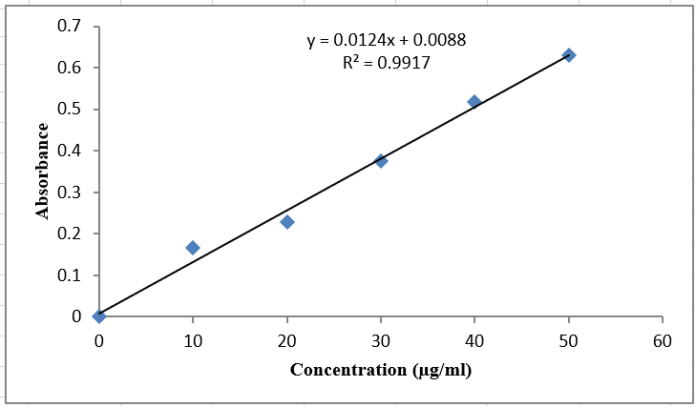

Figure 6: Quercetin calibration curve employed for quantifying flavonoid content

The results of the current study confirm that the extract obtained from the *Cassia tora* plant is rich in flavonoid and polyphenol components, as evidenced by the remarkable decrease in free radicals. This indicates a robust link between using *Cassia tora* to treat various ailments. This discovery, therefore, confirms the effectiveness of *Cassia tora*, which is used by local people worldwide.

CONCLUSION

The local population highly recommends *Cassia tora* for its therapeutic properties in treating various diseases. The hydroalcoholic extract of *Cassia tora* was prepared and used for in vitro testing of the antioxidant properties of its aerial parts. It was found that the IC_{50} values for DPPH, superoxide radicals, hydroxyl radicals, and nitric oxide were reduced, indicating significant antioxidant activity. Moreover, the IC_{50} values of the extract were near to those of the standard drugs. The analysis results showed that the extract contained a significant amount of phenolic and flavonoid compounds. *Cassia tora* extract showed a significant decrease in free radicals in the sample, confirming its strong antioxidant properties. In addition, the study proves that *Cassia tora* contains a significant amount of antioxidant chemicals such as phenols and flavonoids. The current study offers scientists a new opportunity to conduct various in vivo studies to validate the treatment of various diseases. Phytochemistry researchers can identify and characterize the active flavonoids responsible for the antioxidant effect.

FINANCIAL ASSISTANCE

Nil

CONFLICT OF INTEREST

The authors declare no conflict of interest

AUTHOR CONTRIBUTION

Umesh Chaurasia carried out the experimental work and interpreted the results. Vishal Soni supervised and participated in analyzing the results. Ram Kumar Sahu and Vishal Soni drafted the manuscript. All authors reviewed and subsequently approved the final manuscript.

REFERENCES

[1] Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N. Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants*, **8**, (2019)

[2] Jafri SA, Khalid ZM, Khan MZ, Jogezai N. Evaluation of phytochemical and antioxidant potential of various extracts from traditionally used medicinal plants of Pakistan. *Open Chem*, **20**, 1337–1356 (2022)

[3] Jena AB, Samal RR, Bhol NK, Duttaroy AK. Cellular Red-Ox system in health and disease: The latest update. *Biomedicine & Pharmacotherapy*, **162,**114606 (2023)

[4] Edo GI, Samuel PO, Ossai S, Nwachukwu SC, Okolie MC, Oghenegueke O, Asaah EU, Akpoghelie PO, Ugbune U, Owheruo JO, Ezekiel GO. Phytochemistry and pharmacological compounds present in scent leaf: A review. *Food Chem Adv*, **3**, 100300 (2023)

[5] Uzombah TA. The Implications of Replacing Synthetic Antioxidants with Natural Ones in the Food Systems. in *Natural Food Additives* (eds. Prieto, M. A. & Otero, P.) (IntechOpen, Rijeka, 2022). doi:10.5772/intechopen.103810

[6] Tungmunnithum D, Thongboonyou A, Pholboon A, Yangsabai A. Flavonoids and other phenolic compounds

from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, **5**, (2018)

[7] Afolabi OB, Olasehinde OR, Olanipon DG, Mabayoje SO, Familua OM, Jaiyesimi KF, Agboola EK, Idowu TO, Obafemi OT, Olaoye OA, Oloyede OI. Antioxidant evaluation and computational prediction of prospective drug-like compounds from polyphenolic-rich extract of Hibiscus cannabinus L. seed as antidiabetic and neuroprotective targets: assessment through in vitro and in silico studies. *BMC Complement Med Ther*, **23**, 203 (2023)

[8] Rakib M, Ansari AA, Arif M, Ahmad A. A review of phytochemical and biological studies on cassia obtusifolia linn. In folklore medicine of eastern Uttar Pradesh. *World J Pharm Res*, **7**, 191–201 (2018)

[9] Ali MY, Park S, Chang M. Phytochemistry, ethnopharmacological uses, biological activities, and therapeutic applications of Cassia obtusifolia L.: a comprehensive review. *Molecules*, **26**, (2021)

[10] Allegra S, De Francia S, Turco F, Bertaggia I, Chiara F, Armando T, Storto S, Mussa MV. Phytotherapy and Drugs: Can Their Interactions Increase Side Effects in Cancer Patients?. *J Xenobiotics*, **13**, 75–89 (2023)

[11] Chaughule RS, Barve RS. Role of herbal medicines in the treatment of infectious diseases. *Vegetos*, **37**, 41–51 (2023)

[12] Kholiya S, Pandey H, Singh M, Pargaien N, Tiwari A. A comprehensive review of the phytochemical and pharmacological potential of some selected species from Nandhaur landscape, Uttarakhand. *J Pharmacogn Phytochem*, **12**, 131–139 (2023)

[13] Paudel P, Kim DH, Jeon J, Park SE, Seong SH, Jung HA, Choi JS. Neuroprotective effect of aurantio-obtusin, a putative vasopressin V1A receptor antagonist, on transient forebrain ischemia mice model. *Int J Mol Sci*, **22**, (2021)

[14] Garba A, Fakai UR. Proximate, Phytochemical Analysis and Pharmacological Profile of *Cassia torra* Native to Zuru Town, Kebbi State Nigeria. *International Journal of Sustainable Applied Science*, **1(5)**, 525-540 (2023)

[15] Hussain T, Hafiz A, Ravi V, Kumar D, Kazmi I. A Review-Study on Phytochemical and Neuropharmacological Effects of *Cassia tora* Flowers. *Clin Pharmacol Biopharm*, **10(11)**, 1000239 (2021)

[16] Mostafa MM, Ali E, Gamal M, Farag MA. How do coffee substitutes compare to coffee? A comprehensive review of its quality characteristics, sensory characters, phytochemicals, health benefits and safety. *Food Biosci*, **43**, 101290 (2021)

[17] Ko E, Um MY, Choi M, Han T, Kim IH, Shin S. *Cassia tora* seed improves pancreatic mitochondrial function leading to recovery of glucose metabolism. *Am J Chin Med*, **48**, 615–629 (2020)

[18] Nkwocha CC, Felix OJ, Idoko NR. GC-FID spectroscopic analysis and antioxidant activities of methanolic fraction of *Cassia tora* leaves. *Pharmacol Res - Mod Chinese Med*, **9**, 100338 (2023)

[19] Guchu BM, Machocho AK, Mwihia SK, Ngugi MP. In vitro antioxidant activities of methanolic extracts of Caesalpinia volkensii Harms., Vernonia lasiopus O. Hoffm., and Acacia hockii De Wild. *Evid Based Complement Alternat Med*, **2020**, 3586268 (2020)

[20] Hussen EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of Vernonia amygdalina. *BMC Complementary Medicine and Therapies*, **23(1)**, 146 (2023)

[21] Kotha RR, Tareq FS, Yildiz E, Luthria DL. Oxidative stress and antioxidants—A critical review on in vitro antioxidant assays. *Antioxidants*, **11(12)**, 2388 (2022)

[22] Sivagamasundari S. Free Radical Scavenging and Antioxidant Activity of Capparis Zeylanica Linn. *Haya Saudi J Life Sci*, **6(9)**, (2021).

[23] Sasikumar JM, Erba O, Egigu MC. In vitro antioxidant activity and polyphenolic content of commonly used spices from Ethiopia. *Heliyon*, **6**, e05027 (2020)

[24] Shahinuzzaman M, Yaakob Z, Anuar FH, Akhtar P, Kadir NH, Hasan AM, Sobayel K, Nour M, Sindi H, Amin N, Sopian K. In vitro antioxidant activity of Ficus carica L. latex from 18 different cultivars. *Sci Rep*, **10**, (2020)

[25] Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, Chang CM. Determination of antioxidants by DPPH radical scavenging activity and quantitative phytochemical analysis of Ficus religiosa. *Molecules*, **27**, (2022)

[26] Hussen EM, Endalew SA. In vitro antioxidant and free-radical scavenging activities of polar leaf extracts of Vernonia amygdalina. *BMC Complement Med Ther*, **23**, (2023)

[27] Luhata LP, Deng Z, Tanaka E, Usuki T. In vitro DPPH Radical Scavenging Activity of α-Pyrones from Odontonema strictum (Acanthaceae). *J Oleo Sci*, **72**, 571–576 (2023)

[28] Olugbami JO, Gbadegesin MA, Odunola OA. In vitro free radical scavenging and antioxidant properties of ethanol extract of Terminalia glaucescens. *Pharmacognosy Res*, **7**, 49–56 (2015)

[29] Moriasi G, Nelson E, Twahirwa E. In vitro anti-inflammatory, antioxidant and qualitative phytochemical evaluation of the Phytexponent preparation of selected plants. *Adv Tech Biol Med*, **9**, 1–9 (2020)

[30] Sanna D, Fadda A. Role of the hydroxyl radical-generating system in the estimation of the antioxidant activity of plant extracts by electron paramagnetic resonance (EPR). *Molecules*, **27**, (2022)

[31] Hieu LT, Hoa NT, Mechler A, Vo QV. The Theoretical and Experimental Insights into the Radical Scavenging Activity of Rubiadin. *J Phys Chem B*, **127**, 11045–11053 (2023)

[32] Kour R, Sharma N, Showkat S, Sharma S, Nagaiah K, Kumar S, Kaur S. Methanolic fraction of Cassia fistula L. bark exhibits potential to combat oxidative stress and possess antiproliferative activity. *J Toxicol Environ Health A*, **86**, 296–312 (2023)

[33] Mueed A, Shibli S, Al-Quwaie DA, Ashkan MF, Alharbi M, Alanazi H, Binothman N, Aljadani M, Majrashi KA, Huwaikem M, Abourehab MA. Extraction, characterization of polyphenols from certain medicinal plants and evaluation of their antioxidant, antitumor, antidiabetic, antimicrobial properties, and potential use in human nutrition. *Front Nutr*, **10**, (2023)

[34] Kumar RS, Narasingappa RB, Joshi CG, Girish TK, Rao UJ, Danagoudar A. Evaluation of *Cassia tora* Linn. against oxidative stress-induced DNA and cell membrane damage. *J Pharm Bioallied Sci*, **9**, 33 (2017)