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Screening of Secondary Metabolites and their Antioxidant Activity of *Cassia fistula* Linn.

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Abstract: ***Extracts obtained from the dried stem and leaves of Cassia fistula Linn. belonging to Leguminosae family have been tested for screening of secondary metabolites and antioxidant activity. The phytochemical properties of C. fistula has shown a variety of useful properties like blood sugar levels regulation, control of tumor cell proliferation, infertility and inflammation. The results on antioxidant activity indicated that there were higher levels of total phenolics (177.03 mg GAE/g extract), tannins (166.16 mg GAE/g extract), flavonoids (190.17 mg RE/g extract) and DPPH radical scavenging activity in the ethanol extract of the leaf compared to the aqueous ethyl acetate extract. There was a high level of antioxidant activity in the stem ethanol extract (31.28 µg/mL), as well as in the stem aqueous extract (148333 µm TE/g), ferric reducing power leaf ethanol extract (99.07 mM Fe (II) E/mg extract), phosphomolybdenum assay (153.3 AAE/g), and nitric oxide (59.57 %) in the in vitro antioxidant measurements. The antioxidant activity of C. fistula leaf extracts is quite impressive, making them a very good substance for pharmaceutical product development and a potential natural source for new therapeutic agents.***

Keywords: *Cassia fistula,* secondary metabolites, antioxidant activity

1. Introduction

India is revered for its medicinal plants and traditional herbal medicine knowledge. Unlike their international counterparts, Indian traditional healers and practitioners have neither sought Government assistance or personal endeavours to monetize their wealth (Arnao et al., 2000). India's diversified topography and varying agro-climatic conditions support 6000 medicinally relevant indigenous herbal species, making it a rich herbal resource. Only 3000 of the 6000 herbal plants used in traditional, folk, and herbal medicine have been pharmacologically recognized by poor countries, who use 75% of them. Pharmaceutical and other businesses have searched for suitable natural items for medication research and discovery for 20 years (Barua et al., 2014).

Indian ethnobotanical resources come from 227 ethnic groups and 550 tribes. Indian tribes and rural people in numerous locations depend on medicinal plants for their primary healthcare. Despite the ambiguity of ethnomedicinal plant knowledge, active research continues, attracting global scientists and academics to research medicinal plants for disease solutions. Scholars in Tamil Nadu, India have documented the ethno botanical values of different medicinal plants with the help of tribal and rural populations. Ethnobotanical drug development has gained attention in this new area for biomedical applications (Rajan et al., 2002).

Certain phytoconstituents called ‘secondary metabolites’ are playing important role in drug development since ancient times. They are natural compounds which occur incidentally and do not directly affect the plant growth, development, or reproduction. Plant scientists paid little attention to these chemical compounds since they were physiologically unimportant (Cook and Samman, 1996). These secondary metabolites have attracted interest in recent decades due to their potential involvement in human nutrition, cosmetics, medicines, and plant defense mechanisms, which can protect human health when eaten as medications (Deshpande et al., 2007).

Synthetic antioxidants such as BHT, n-Propyl Gallate (PG) and BHA have been widely used in oxidation systems despite their liver damage and cancer risks (Flieger et al., 2021). Mild synthetic antioxidants with low solubility and health risks were used to enhance studies. Some synthetic antioxidants are harmful. Recently, food-derived antioxidants have received attention for their potential to stabilize food against oxidation (Siddhuraju and Becker, 2003). Vegetable antioxidants such phenolic acids, flavonoids, carotenoids, tocopherol, and ascorbic acid reduce oxidative damage and improve food quality (Roginsky and Lissi, 2005). Recent research has investigated several plant species with novel antioxidants to reduce free radical damage and tissue damage. Similarly, phenolic-rich plant species are sought for their antioxidant properties (Zhishen et al., 1999).

2. Materials and Methods

Quantification assays

Determination of total phenolic and tannin contents

The total phenolic content was [10]. In test tubes with distilled water, we diluted extracts to 1 mL. Each tube received 0.5 mL of folin-ciocalteu phenol reagent (1:1 with water) and 2.5 mL of 20% sodium carbonate solution. After vortexing the reaction mixture, the test tubes were darkened for 40 min and measured at 725 nm against the reagent blank. Trios produced gallic acid equivalents. Polyvinyl polypyrrolidone-treated extracts evaluated tannins. In a test tube, 100 mg PVPP, 1.0 mL distilled water, and tannin with phenolic extract were added. After vortexing, store the content at 4ºC for 4 hours. Centrifuged at 3000 rpm for 10 min at room temperature, supernatant was collected. Although PVPP precipitated tannins, this supernatant contains solely

phenolics. The supernatant was tested for non-tannin phenolics. The sample's tannin concentration was calculated as% tannin = total phenolics – non-tannin.

Estimation of flavonoid content

Aassessed sample extract flavonoid concentration using a significantly modified colorimetric technique. Mixing 0.5 mL extract, 2 mL distilled water, and 0.15 mL 5% $NaNO_2$. Add 0.15 mL of 10% AlCl3 after 6 min and let stand. Add 2 mL 4% NaOH to the mixture. It was carefully combined and left for 15 min after adding distilled water to 5 mL. Compared to reagent blank, mixture absorbance was 510 nm. Rutin was used to measure total flavonoids. Every figure was mg of RE per gram of extract.

***In vitro* antioxidant assays**

Freeradical scavenging activity on DPPH•

DPPH radicals measure antioxidant activity quickly. Radical-scavenging antioxidants change purple to yellow. Experimental DPPH decrease IC50 was obtained. Thus, antioxidant activity was shown by decreased IC values. The extracts to DPPH for antioxidant activity. Methanol was used to dilute various sample concentrations to 100 µL. Mix in 5 mL 0.1 mM DPPH methanol. Set tubes at 27°C for 20 minutes. Absorbance 517 nm.

Antioxidant activity by the ABTS assay

Sample antioxidant activity was determined. ABTS radical cation decolorization. ABTS was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulfate in the dark at room temperature for 12-16 hours. The solution, diluted in ethanol (1:89 v/v) and equilibrated at 30○C, showed an absorbance of 0.700 ± 0.02 at 734 nm during testing. We diluted the sample extract stock solution to achieve a 20% to 80% blank absorbance blockage in 10 µL aliquots. In ethanol, mix 1 mL diluted ABTS solution with 10 µL sample or Trolox standards (0-15 µM). Check absorbance at 30○C 30 minutes after mixing. These tests employed solvent blanks. To calculate 734 nm inhibition in %, each standard dilution was tested three times. Normal trolox concentration graphs follow. TOA is evaluated in µM/g of sample extract as the trolox concentration with similar antioxidant activity.

Ferric-reducing/antioxidant power (FRAP) assay

TPTZ–Fe(III) complex was reduced to TPTZ–Fe(II) to test extract antioxidant capability. Ferric-TPTZ-reducing chemical is 1 mM Fe. Phenolic extract antioxidant capacity. FRAP reagent (900 µL) was incubated at 37○C with 90 µL distilled water and 30 µL test sample or methanol (for blank). We incubated reagent blank and test samples in a water bath at 37○C for 30 minutes. Sample diluted 1/34 with response cocktail. According to Siddhuraju (2003) Studies on antioxidant activities of *Mucuna* seed the FRAP reagent contains 2.5 mL of 20 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM FeCl3.6H2O, and 25 mL of 0.3 M acetate buffer (pH Incubation yielded 593 nm spectrophotometer absorbance. A calibration curve was established using Fe (II) methanolic solutions (FeSO4.7H2O) at concentrations from 100 to 2000 µ. The antioxidant concentration with ferric-TPTZ-reducing activity was 1 mM FeSO4.7H2O. EC increased FRAP absorbance to 1 mM Fe (II) solution, according to the regression equation.

Phosphomolybdenum assay

The antioxidant activity of the samples was tested by creating green phosphomolybdenum complexes. One millilitres of reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was combined with 100 microliters of sample solution containing 1 mM dimethyl sulphoxide. The vials with caps were placed in a water bath and incubated at 95○C for 90 minutes. Upon reaching room temperature, the absorbance of the mixture was assessed at 695 nm in comparison to a blank. Data on the antioxidant activity, expressed as a mean value per 100 grams of extract, is presented in grams.

Assay of superoxide radical scavenging activity

In the riboflavin-light-NBT system, several extracts were used to reduce formazan formation by scavenging superoxide radicals. Each 3 mL portion should be supplemented with 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, 12 mM EDTA, 0.1 mg NBT, and 100 µL of sample solution. The reaction was initiated by exposing the reaction mixture to sample extract for 90 seconds; thereafter, the absorbance was measured at 590 nm. The reaction assembly was housed in an aluminum-foil container. A dark reaction mixture was placed in identical tubes to serve as a blank. Where A0 is the absorbance of the control and A1 is the absorbance of the sample extract or standard, the formula for the percentage suppression of superoxide anion formation.

Assay of nitric oxide scavenging activity

Nitric oxide was generated spontaneously by reacting sodium nitroprusside in an aqueous solution with oxygen at a physiological pH.At the end of the result nitrite ions weremeasured using the Greiss reagent. Nitric oxide scavenger's lower nitrite ion production by competing with oxygen. The experiment involved incubating a 100 µL sample solution of different extracts at room temperature for 150 minutes with 10 mM sodium nitroprusside in phosphate buffered saline (0.2 M, pH 7.4). The reaction mixture without the sample served as the control. There was an addition of half a milliliter of Greiss reagent (1% sulfanilamide, 2% H3PO4, 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) following incubation. The absorbance at 546 nm was 16.0% for the synthesized chromophore.

3. Results and Discussion

Total phenolics, tannins and flavonoid contents

Table 1 shows comparison of solvent extracts, ethanol extracts of *C. fistula*leaves and stems total phenolic and tannin compounds. Total phenolic (177.03 and 149.58 mg GAE/g extract, respectively) and tannin contents were higher in *C. fistula* ethanol extracts of stems and leaves. *C. fistula*ethyl acetate leaf and stem extracts had the greatest flavonoid concentration (169.84 and 190.17 mg RE/g extract, respectively), while the aqueous leaf extract had the lowest (11.72 mg RE/g extract). The structural chemistry of many phenolic compounds makes them effective free radical scavengers. This includes plant polyphenols. Antioxidant

capabilitiesare conferred by polyphenols by virtue of their high reactivity as hydrogen or electron donors, stability and delocalization of unpaired electrons, and capacity to bind metal ions. By scavenging free radicals, blocking enzymes that generate free radicals, and chelating metal ions (such as copper and iron), flavonoids function as antioxidants. The structural makeup of flavonoids determines their ability to scavenge nearly all reactive oxygen species (ROS). The antioxidant activity of phenolic compounds has been associated with several research.

Table 1: Total phenolics, tannin and flavonoid contents of *C. fistula*

Sample	Solvents	Total Phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)	Flavonoids (mg RE/g extract)
Leaf	Ethyl acetate	52.66 ± 0.48	47.85 ± 0.38	169.84 ± 1.03
	Ethanol	177.03 ± 1.28	166.16 ± 1.56	47.14 ± 0.22
	Aqueous	134.45 ± 2.91	135.3 ± 3.17	11.72 ± 0.14
Stem	Ethyl acetate	154.9 ± 0.48	149.89 ± 0.68	190.17 ± 0.19
	Ethanol	149.58 ± 1.45	136.79 ± 1.48	25.58 ± 0.22
	Aqueous	88.23 ± 0.84	81.1 ± 0.72	16.91 ± 0.12

GAE - Gallic Acid Equivalents& RE - Rutin Equivalents
Values are mean of triplicate determination (n=3) ± standard deviation; statistically significant at $p < 0.05$ where $^{a}>^{b}>^{c}$ in each column

***In vitro* free radicals scavenging activities**

DPPH scavenging activities

Fig. 1 shows that *C. fistula* extracts may scavenge DPPH radicals. As control variables, this study included both synthetic BHT and the naturally occurring antioxidant rutin. Out of all the solvent extracts, ethanol showed the most activity. The DPPH radical scavenging activities of the stem (IC_{50}: 60.33 µg/mL) and leaf (IC_{50}: 68.82 µg/mL) ethanol extracts were on par with the standards BHT (IC_{50}: 22.24 µg/mL) and rutin (IC_{50}: 17.57 µg/mL). Aqueous extracts were shown to have reduced free radical scavenging activity. One way to quickly and accurately determine whether a material has antioxidant potential is to use the DPPH radical scavenging assay. Since DPPH can undergo a stable diamagnetic transformation either by taking an electron or a radical, the theory suggests that the test is substrate polarity independent. Spectrophotometric monitoring at 517 nm. The purple solution turns yellow as a result of free radical scavenging. Because smaller molecules have easier access to the DPPH radical site, they appear to have a higher antioxidant capacity. This suggests that the extracts may contain smaller compounds instead of larger ones.

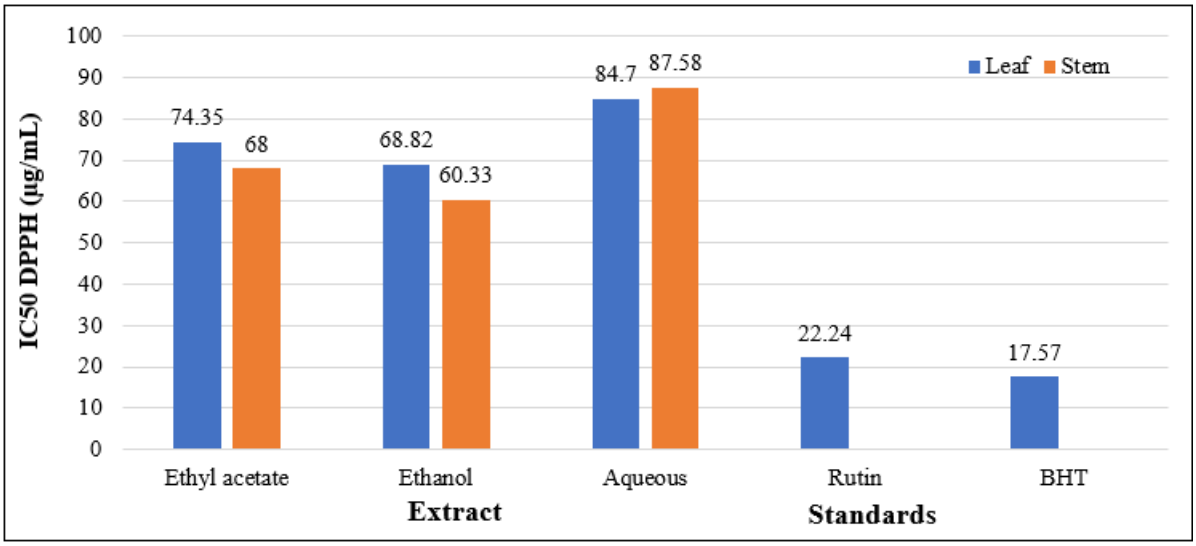

Figure 1: DPPH radical scavenging activity of *C. fistula*leaf extracts

ABTS scavenging activities of*Cassia fistula*

The ability of *C. fistula* leaf and stem extracts to scavenge ABTS radicals is shown in Fig. 2. Both of the *C. fistula* aqueous extracts were determined to be the most efficient ABTS radical scavengers in this experiment. With measurements of 142083 and 148333 µMTE/g extract, respectively, the aqueous extracts of the leaves and stems of *C. fistula* showed the greatest scavenging capabilities. By comparison, the synthetic antioxidant BHT (156944.4 µM TE/g) and the traditional natural antioxidant rutin (155468.8 µM TE/g) showed slightly better efficacy.The ABTS radical can evaluate the antioxidant capability of hydrophilic and lipophilic compounds in test materials, is soluble in organic and aqueous solvents, and is unaffected by ionic strength. The radical is also a good choice for measuring phenolics' antioxidant capacity because of their modest redox potentials (0.68 V). This thermodynamic property allows for the interaction of the ABTS radical with a wide variety of phenolic compounds. Molecular weight, the number of aromatic rings, and the type of hydroxyl group substitutions have a greater impact on the effectiveness of high molecular weight phenolics (tannins) in quenching free radicals (ABTS) than do particular functional groups, according to reports. The results of the ABTS experiment showed that the

extracts of *C. fistula* can donate a lot of hydrogen and maybe even scavenge free radicals; when taken with other nutrients, they can operate as main antioxidants.

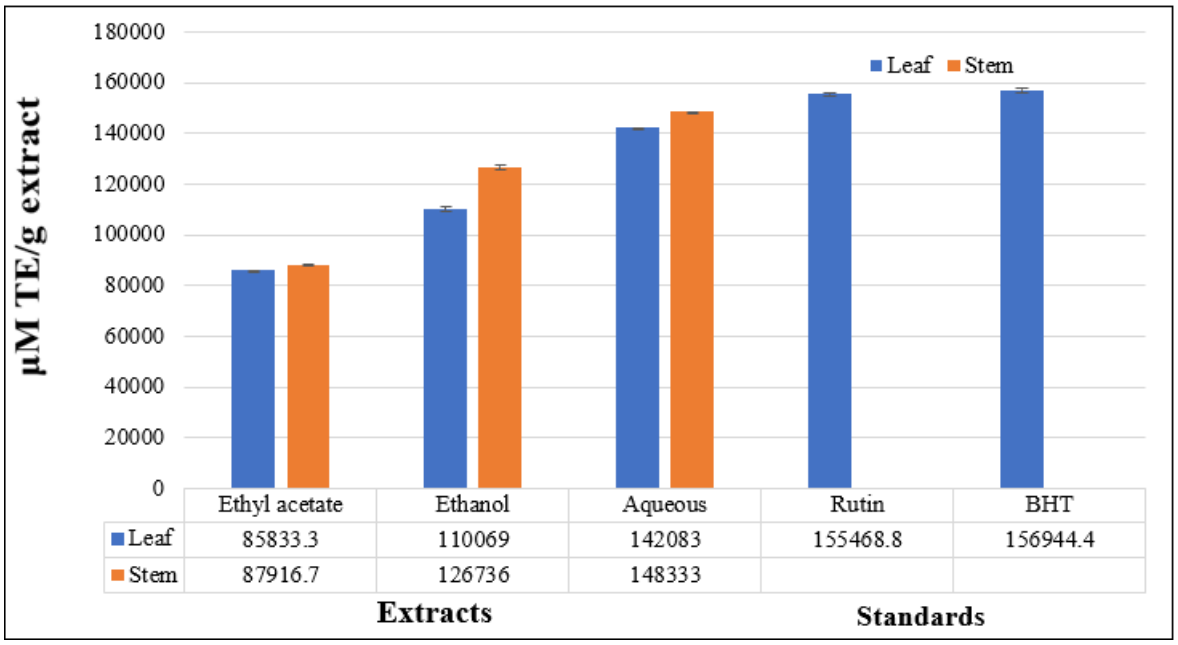

Figure 2: ABTS cation radical scavenging activity of *C. fistula*leaf extracts

FRAP assays

Fig. 3 shows the results of the ferric reducing antioxidant power (FRAP) test on extracts from leaves and stems of *C. fistula*. Out of all the solvent extracts tested, the two with the highest activity were the ethanol (99.07 mM Fe(II)/mg extract) and the ethyl acetate (86.17 mM Fe (II)/mg extract) leaf extracts. Other areas showed ferric reduction capacity that was moderate. A material's antioxidant capacity in the reaction medium can be measured using the ferric reducing antioxidant power test, which is based on its reducing ability. The correlation between antioxidant activity, namely in free radical scavenging and the reducing capacity of bioactive chemicals, such as low and high molecular weight phenolics, was demonstrated in. The in vitro ferric-reducing antioxidant power assay has also shown that potential antioxidants increase blood plasma's total antioxidant capacity, which is the presence of compounds in the ethanol extracts of *C. fistula* suggests that they have a considerable affinity for ferrous ions, which allows them to quench or scavenge them via redox reactions. This is supported by their ferric reducing ability.

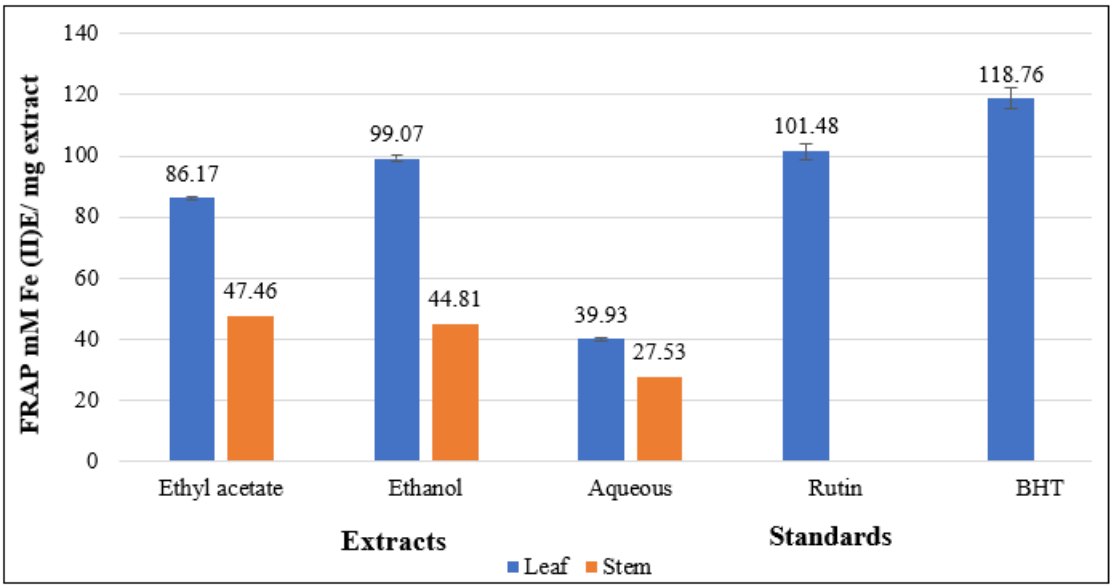

Figure 3: Ferric reducing antioxidant power activity of *C. fistula*leaf extracts

Phosphomolybdenum assay

Several solvent extracts from *C. fistula* leaves and stems were tested for total antioxidant capacity, and the results are shown in Fig. 4. In most solvents, the leaf extract was more active than the stem extract. When compared to other solvent extracts, the phosphomolybdenum reduction is maximum in the ethanol leaf extract (153.3 mg AAE/g extract). A wide range of extract components have

antioxidant properties, from 57.59 to 129.61 mg AAE/g extract. The phosphomolybdenum test is based on the ability of plant extracts to convert molybdenum (VI) to molybdenum (V), which causes a green phosphate/Mo(V) complex to form in an acidic pH setting. We performed the assay on plant extracts since it is simple and doesn't interfere with other commonly used antioxidant tests. It is possible to correlate the total antioxidant capacity with the free radical scavenging activity of *C. fistula* preparations by measuring their antioxidant activity in ascorbic acid equivalents. The process by which *C. fistula* extracts from the leaves and stems convert Mo (VI) to Mo (V) could be due to electron transfer or hydrogen ion transfer, both of which are supported by the bioactive chemicals present in these parts of the plant. This is especially true of the phenolics and flavonoids.

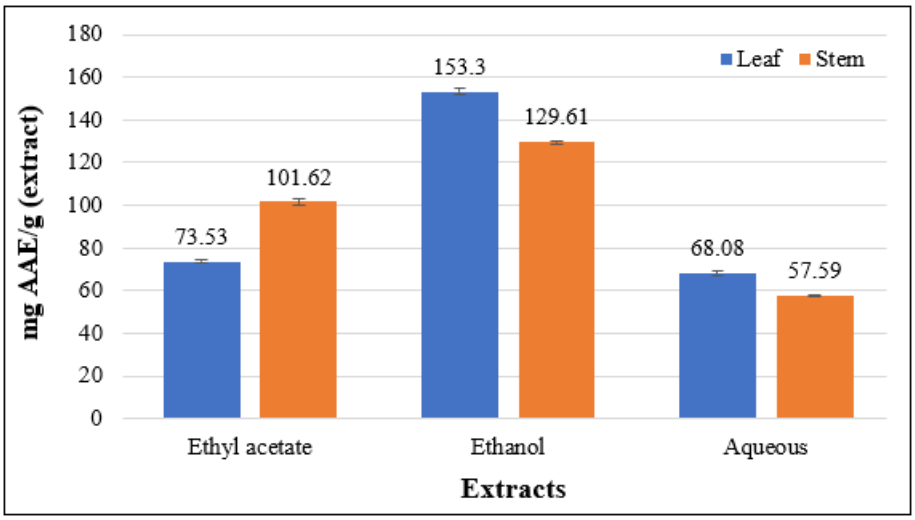

Figure 4: Phosphomolybdenum assay of *C. fistula*leaf extract

Nitric oxide radical scavenging activity

Fig. 5 displays the nitric oxide radical scavenging activity of various solvent extracts from all the samples. The ethanol extract of the leaves had the second-highest free radical scavenging activity at 57.39%, while the water-based extract had the highest at 59.57%. The ethyl acetate stem extract had the lowest radical scavenging activity at 30.57%.

Inflammation, cancer, and other pathological disorders can be linked to reactive nitrogen species, which are generated when reacting with oxygen or superoxides. These species are particularly reactive. The structure and functional behavior of numerous cellular components is influenced by these chemicals. Because phytochemicals have the ability to inhibit NO creation, they could be of great use in protecting the human body from the harmful consequences of NO overload. In addition, the scavenging activity has the potential to halt the harmful cascade of events that occurs when NO is produced in excess. The increased scavenging activity observed in the aqueous and ethanol extracts of *C. fistula* leaves and stems makes it evident that this plant can be utilized to mitigate the harmful effects of reactive nitrogen species in humans.

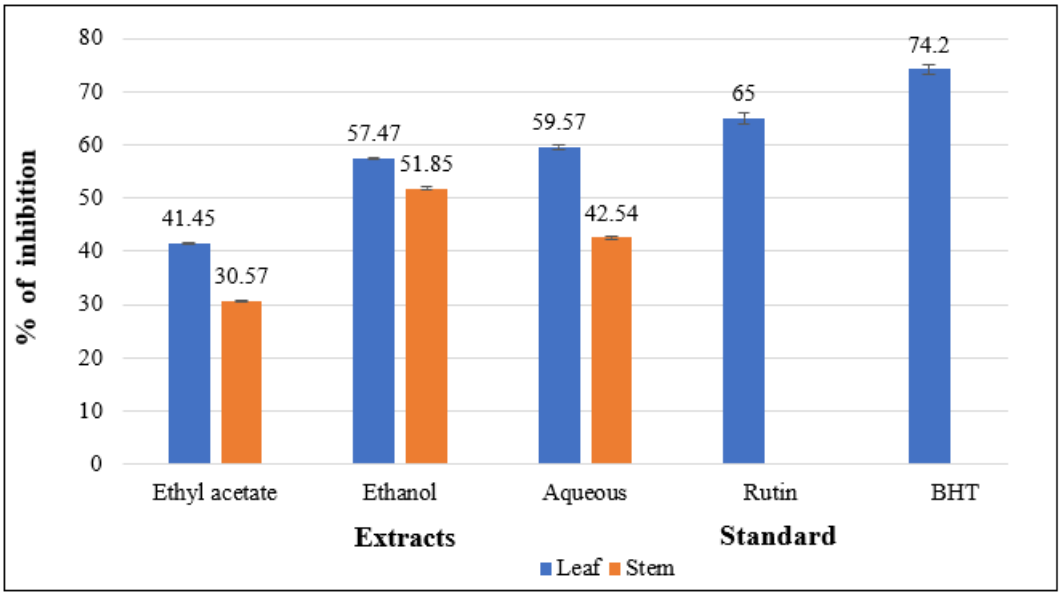

Figure 5: Nitric oxide radical scavenging activity of *C. fistula* leaf extract

Finally, *C. fistula* shows promising result as an antioxidant food additive to replace synthetic antioxidants, due to its potent antioxidant properties. Accordingly, the study establishes the value of commonly used fruit, which might be highly relevant to the development of new drugs. In order to find a reliable and inexpensive medicine that can help people, more studies are needed to isolate and identify particular bioactive compounds and to understand how they work as antioxidants, antibacterials and anti-inflammatory agents.

References

[1] Arnao, M.B., Cano, A., and Acosta, M. (2000). A method to measure antioxidant activity in organic media: application to lipophilic vitamins. *Redox Rep*. 5(6), 365-70.

[2] Barua C.C., Sen S., Das A.S., Talukdar A., JyotiHazarika N., Barua A., Barua I. A comparative study of the in vitro antioxidant property of different extracts of Acoruscalamus Linn. J. Nat. Prod. Plant Resour. 2014; 4: 8–18

[3] Beauchamp, C., and Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gels. *Analytical Biochemistry*., 4, 276-277.

[4] Cook, N., and Samman, S. (1996). Flavonoids? Chemistry, metabolism, cardioprotective effects, and dietary sources. *Journal of the European Ceramic Society.*, 66–76.

[5] Deshpande, Y.S., Dhalwal, K., Purohit, A.P.(2007). Evaluation of in vitro antioxidant activity of,*Sidarhombifolia* (L.) ssp. retusa (L.). *J Med Food.,* 10(4), 683-688.

[6] Flieger, J., Flieger, W., Baj, J., and Maciejewski, R., Antioxidants (2021). Classification, Natural Sources, Activity/Capacity Measurements, and Usefulness for the Synthesis of Nanoparticles. *Materials* (Basel)., 14(15), 4135.

[7] Garcia, O., Castillo, J.,Lorente, J., Ortuño, A., and Del Rio, J. (2000). Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chemistry.,* 68(4), 457–462.

[8] Hagerman, A.E., Riedl, K.M., Jones, G.A., Sovik, K.N., Ritchard, N.T., Hartzfeld, P.W., and Riechel, T.L. (1998). High molecular weight plant polyphenolics (Tannins) as biological antioxidants. *Journal of Agricultural Food Chemistry*., 46**,** 1887−1892.

[9] Kumarappan, C. T., Thilagam, E., and Mandal, S.C, (2012). Antioxidant activity of polyphenolic extracts of Ichnocarpusfrutescens. *Saudi Journal of Biological Sciences,* 19 (3), 349–355.

[10] Luo, D., and Fang, B. (2008). Structural identification of ginseng polysaccharides and testing of their antioxidant activities. *Carbohydrate Polymers*., 72(3), 376–381.

[11] Mak, Y.W., Chuah, L.O., Ahmad, R., and Bhat, R. (2013) Antioxidant and antibacterial activities of hibiscus (*Hibiscus rosa-sinensis* L.) and Cassia (*Sennabicapsularis* L.) flower extracts. *Journal of King Saud University – Science.*,25(4), 275–282.

[12] Prieto, P., Pineda, M., and Aguilar, M. (1999). Spectrophotometric quantitative of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry.,*269, 337–341.

[13] Pulido, R., Bravo, L., and Sauro-Calixto, F. (2000) Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/antioxidant power assay. *Journal of Agricultural and Food Chemistry*., 48, 3396-3402.

[14] Prior, R.L., Wu, X., and Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem.* 53(10), 4290-302.

[15] Rice-Evans, C.A., and Miller, N.J. (1997). Factors influencing the antioxidant activity determined by the ABTS. + radical cation assay. *Free Radic Res.* 26(3), 195-9.

[16] Re, R., Pellegrini, N., Proteggente, A., Pannala, A., ang, M., and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine.,*26, 1231–1237.

[17] Roginsky, V., and Lissi, E. (2005). Review of methods to determine chain-breaking antioxidant activity in food. *Food Chemistry*, 92(2), 235–254.

[18] Rajan, I., Rabindran, R., Jayasree, P.R andKumar, P.R,. (2014). Antioxidant potential and oxidative DNA damage preventive activity of unexplored endemic species of Curcuma. *Indian J Exp Biol.,* 52(2),133-138.

[19] Sreejayan Rao, M.N. (1997). Nitric oxide scavenging by curcuminoids. *J Pharm Pharmacol.* 49(1):105-107.

[20] Siddhuraju, P., Mohan, P., and Becker, K. (2002). Studies on the Antioxidant Activity of Indian Laburnum (*Cassia fistula* L.): A Preliminary Assessment of Crude Extracts from Stem Bark, Leaves, Flowers and Fruit Pulp. Food Chemistry, **79**, 61-67.

[21] Serafini, M., Bugianesi, R., and Maiani, G. (2003). Plasma antioxidants from chocolate. *Nature* 424, 1013.

[22] Siddhuraju, P., and Becker, K. (2003). Studies on antioxidant activities of *Mucuna* seed (*Mucuna pruriens* var. *utilis*) extracts and certain non-protein amino acids through *in vitro* models. *Journal of the Science of Food and Agriculture.,*83,1517-1524.

[23] Sarwar, R., Farooq, U., Khan, A., Naz, S., Khan, S., Khan, A., Rauf, A., Bahadar, H, and Uddin, R.(2015). Evaluation of Antioxidant, Free Radical Scavenging, and Antimicrobial Activity of *Quercusincana*Roxb. *Front Pharmacol*.6, 277.

[24] Tepe, B.,Sokmen, M., Akpulat, H.A., and Sokme, A. (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. *Food Chemistry*, 95(2), 200–204.

[25] Velioglu, Y.S., Mazza, G., Gao, L., and Oomah, B.D. (1998). Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables, and Grain Products.

Journal of Agricultural and Food Chemistry., 46(10), 4113–4117.

[26] Valko, M., Leibfritz, D., Moncol, J.,Cronin, M.T., Mazur, M., and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol.* 39(1), 44-84.

[27] Yen, G.C., Duh, P.D., and Tsai, C.L. (1993). Relationship between antioxidant activity and maturity of peanut hulls. *Journal of Agricultural and Food Chemistry*, 41(1), 67–70.

[28] Yıldırım, A., Mavi, A., and Kara, A.A. (2001). Determination of Antioxidant and Antimicrobial Activities of *Rumex crispus* L. Extracts. *Journal of Agricultural and Food Chemistry*,49(8), 4083–4089.

[29] Yen, M.T., Yang, J.H., and Mau, J.L. (2008). Antioxidant properties of chitosan from crab shells. *Carbohydrate Polymers*, 74(4), 840–844.

[30] Zhishen, J., Mengcheng, T., and Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on super oxide radicals. *Food Chemistry*.,64, 555-559.