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PHYTOCHEMICAL, PHARMACOGNOSTIC INVESTIGATION AND ANTIOXIDANT ACTIVITY OF *CASSIA FISTULA* LINN. (AMALTAS)

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ABSTRACT

Cassia fistula Linn. which belongs to family Leguminosae is a medium-sized tree and its different parts are used in ayurvedic medicine as well as home remedies for common ailments. Sequential extraction was carried out using solvents viz. petroleum ether, chloroform, ethanol and water from barks of stem of the plant and investigated for preliminary Phytochemical Screening, P'cognostical features and Biological property/ antioxidant activity. Evaluation of Phytochemicals such as alkaloids, flavonoids, carbohydrates, glycosides, protein and amino acids, saponins, and triterpenoids revealed the presence of most of constituents in polar extracts (ethanol, methanol, and aqueous) compared with nonpolar extracts (petroleum ether and chloroform). Furthermore, the ethanol extract possess best antioxidant potential and property as compared to standard ascorbic acid with IC 50value 15-16µg/ml.

KEYWORDS: *Cassia fistula*, Amaltas, Antioxidants, DPPH, IC 50 Value etc.

INTRODUCTION

Traditional medicines using herbal drug exist in every part of the world. Even as we entered into the new century with its exciting prospect of gene therapy, herbal medicines remain one of the common form of the therapy available to the world population. In the view of the progress of western medicines not only new synthetic drugs but also herbal drugs have to fulfill the international requirement on quality, safety and efficacy. Herbal drugs have an advantage of being available for patient in the geographical area of the specific traditional medicines. The development procedure of herbal drugs for worldwide use to be different from that of synthetic drugs. The basic uses of Plants in Medicine will continue in the Future,

as a Source of therapeutic agents, and as raw material base for the Extraction of Semi Synthetic chemical Compounds such as Cosmetic, Perfumes and food industries.[1-5]

Oxidative stress occurs when the production of free radicals goes beyond the protective defenses in the body. Oxidative stress and free radical damage to cells may initiate the early stages of cancer and heart disease. The human body is not without its own defenses against this damage. It creates many different types of molecules -- Antioxidants -- to combat these free radicals and protect the cells from attack by oxygen. Antioxidants can safely interact with free radicals and stop the chain of damaging reactions before damage is done to cells.[6-8]

MATERIALS AND METHODS

Plant Material

The Plant species *Cassia fistula* Linn. (Amaltas) was selected for this Research project work. For this purpose, barks of stem of this plant species collected from central nursery, Forest Research Institute (FRI) Dehradun and authenticated from Department of Life sciences, Shri Guru Ram Rai Institute of Technology & Science (SGRRITS) Dehradun. A Voucher specimen Herbarium of this plant was also kept in this department of college.

Cassia fistula Linn. is widely grown as an ornamental plant in tropical and subtropical areas. It blooms in late spring. Flowering is profuse, with trees being covered with yellow flowers, many times with almost no leaf being seen. It will grow well in dry climates. Growth for this tree is best in full sun on well-drained soil. The tree has strong and very durable wood.[9,10]

Oxyanthraquinone, dihydroxyanthraquinone phytochemicals were specially found in the barks of this plant species and various traditional uses of this plant species are in constipation, cough and cold, intestinal disorders and skin disorders.[11-14]

STANDARDIZATION PARAMETERS

PHARMACOGNOSTIC INVESTIGATION/ORGANOLEPTIC FEATURES

Color : Dark brown.

Odor : Odourless.

Taste : Slight & acrid.

Texture : Rough surface.

Determination of the Foregin Matter

Foreign Matter in herbal drugs Consists of either Parts of the Medicinal Plant or it May be any Organism, Part of Product of an Organism. It may also Include minerals Admixture not adhering to the medicinal Plant material e.g., soil, stone, dust etc. The specified of quantity of plant material is spread on a thin layer of paper. By visual inspection or by using a magnifying lens (5X or 10 X), the Foreign matters are Picked out and the percentage are Picked out the Percentage is Recorded.[15,16]

Determination of Physical Constants

Ash Values

Used to determine quality and purity of a crude drug and to establish the identify of it. Ash Contains inorganic radicals like Phosphates, carbonates and silicates of sodium, Potassium, Magnesium, calcium etc. These are Present in definite amount in a particular Crude drug hence, quantitative determination in terms of various ash values helps in their standardization.[17,18]

Extractive Values

Alcohol Soluble Extractive Value

Weigh about 4gm of the coarsely powdered drug in a weighing bottle and Transfer it to a dry 250ml conical flask. Fill a 100ml graduated Flask to the delivery mark the solvent (90% alcohol). Wash out the weighing bottle and Pour the washings, together with the solvent into the conical flask. Cork the flask and set aside for 24 hours, shaking frequently. (Maceration) Filter into a 50ml Cylinder. When Sufficient Filtrate has cololected, transfer 25ml of the filtrate to a weighed, thin porcelain dish, as used for the ash values determinations. Evaporate to Dryness on a water–bath and complete the drying in an oven at 105ºC for 6 hrs. Cool in a desiccator for 30minutes and weigh immediately. Calculate the percentage w/w of Exctractive with reference to the air – dried- drug.

Water Soluble Extractive Value

Steps are similar as said in above procedure except using Chloroform Water instead of alcohol.[19-22]

Extraction

The Powered barks of *Cassia Fistula* was Extracted with 95% v/v alcohol by hot Percolation Method, Separately. Aqueous extracts were also Prepared by using Chloroform water I.P using Maceration Process.

Successive Solvent Extraction

About 150gm of dried Powder of Barks of *Cassia fistula* was extracted with Solvent of different Polarity in succession, Starting with a highly non polar solvent (Pet ether 60-80ºC) followed by comparatively Less non polar Solvent (Chloroform) and Finally with a more Polar Solvent (Ethanol). Extracts were Subjected to Preliminary Qualitative Phytochemical Screening or Investigation.[23-26]

Qualitative TLC Analysis

The AlcE showed the Presence of flavonoids the details of TLC is as Follow

Adsorbent: Silica gel GF 254 (activated).

Thickness: 0.4mm.

Plate size: 10* 20cm.

Activation temp: 110ºC for 1hr.

Volume of spot: 20µl.

Solvent system: Ethyl acetate, Formic acid, Glacial acetic acid, water (1: 011: 0.11:0.27).

The Retardation factor (R f) is calculated using following formula

$$Rf = \frac{\text{Distance travelled by solute from the origin}}{\text{Distance travelled by solvent from the origin}}$$ [27-30]

Antioxidant activity

A rapid, simple and inexpensive method to measure antioxidant capacity of plant species involves the use of the free radical, 2,2- Diphenyl -1-1 Picryl hydrazyl (DPPH).

DPPH is widely used to test the ability of compound to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity. It has also been used to quantity antioxidants in complex biological system in recent years. The DPPH method can be used for soild or Liquid samples and is not specific to any Particular antioxidant Component, but applies to the overall antioxidant capacity of the sample.[31-35] A measure of total antioxidant

capacity helps understood the functional Properties of compound. An easier way to present antioxidant activity of compound would be to reference a common reference standard

% Inhibition = (Abs control-Abs test) /Abs control *100

It is a simple method that has been developed to determine the antioxidant activity of Compound utilizes the stable 2, 2 - diphenyl -1- picryl hydrazyl (DPPH) radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517nm and is purple in color.[36,37,38] The color turns from purple to yellow as the molar absorbtivity of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes Paired with hydrogen from a free radical scavenging antioxidant to from the reduced DPPH –H. The resulting depolarization is Stoichiometric with respect to number of electrons captured.[39-42]

RESULTS AND DISCUSSION

Table No. 01: Observation of Physiochemical Parameter of the Barks of *Cassia fistula*

S.N.	ORGANOLEPTIC	EVALUATION	OBSERVATION
1.	Parameters	Nature Colour Odour Taste	Rough Brown Odourless Tasteless
2.	Physiochemical Evaluation	% Loss of drying % total ash value % Water soluble ash value % acid insoluble ash value	17.2 % 7.5% 0.025% 1%
3.	Extractive value	% alcohols soluble Extractive value % Water soluble Extractive value	20% 30%

The Preliminary Phytochemical tests revealed that Barks Contained Carbohydrate, Glycosides, Flavonoids, protein, Tannins and Phenol Compounds.

Table No: 02 Phytochemical Screening Results.

S. No	PHYTOCHEMICAL CONSTITUENT	CHEMICAL TEST	EXTRACTS			
			PEE	CE	ALCE	AQE
1.	Carbohydrate test	Molish's test	-	-	+	+
		Benedict's test	+	+	+	+
		Barfoed's test	-	+	+	+
		Fehling test	-	-	-	+
2.	Alkaloids	Mayer's test	-	+	+	+
		Hager's test	+	-	-	+
		Dragendroff's test	+	+	+	+
		Wagner's test	+	+	+	+

3.	Flavonids	Shinoda test	+	-	-	-
		Alkaline test	-	+	-	+
4.	Glycosides	Baljet's test	+	-	-	+
		Raymond's test	-	-	-	+
		Bromine water test	+	-	+	-
5.	Proteins	Millon's test	-	+	+	+
		Biuret test	+	+	+	-
		Xanthoproteic test	+	+	+	+
6.	Tannis and Phenol Compounds	5% $Fecl_3$ Solution	-	-	-	+
		Bromine Water	+	+	+	-
		Acetic acid solution	-	+	-	+
		Dilute Iodine Solution	+	+	+	-
		LeadAcetate Solution	+	-	-	-
7.	Steroids	Salkowski test	+	-	-	+
		Sulphur test	+	-	-	+

Table No. 03: Absorbance v/s Concentration in µg/ml. for various extracts of *Cassia fistula* .

Conct^{n} (µg/ml)	Ascorbic acid (standard)	Pet. ether extract	Chloroform extract	Alcoholic extract
5	0.2813	0.2283	0.178	0.2544
10	0.2767	0.1606	0.1847	0.1777
15	0.1839	0.1921	0.2676	0.1428
20	0.1315	0.1504	0.2245	0.127
25	0.1052	0.193	0.1514	0.1254
30	0.041	0.1008	0.1549	0.053

Control Value: 0.281

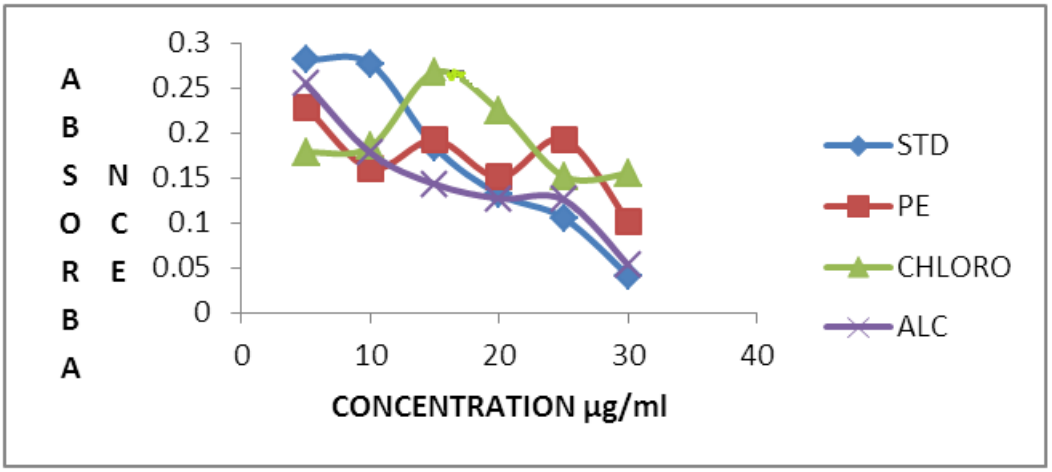

Figure No. 01: Absorbance v/s Concentration of extracts.

Formula for % Inhibition = (Abs control - Abs sample) / Abs control × 100

Table No. 04: % Inhibition v/s Concentration in µg/ml. for various extracts of *Cassia fistula* Linn.

Conct^{n} (µg/ml)	Ascorbic acid (standard)	Pet. ether extract	Chloroform extract	Alcoholic extract
5	0.177	18.98	36.83	9.72
10	1.809	43.09	34.45	36.94
15	34.74	31.83	5.04	49.32
20	53.33	46.62	20.33	54.93
25	62.66	31.51	46.27	55.5
30	85.45	64.22	45.03	81.19

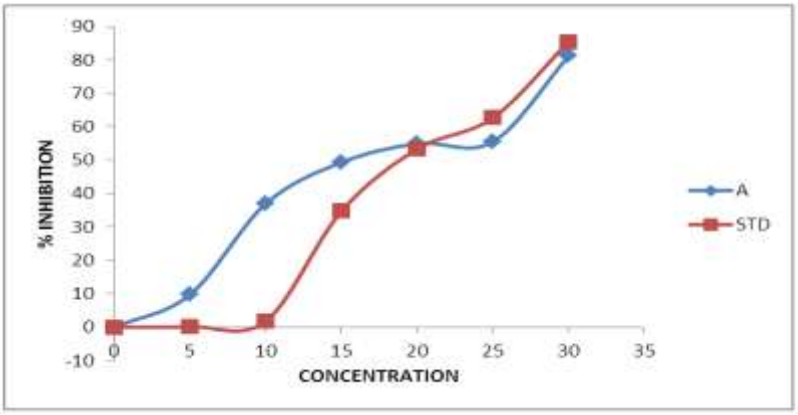

Figure No. 02: % Inhibition v/s Concentration of extracts.

Natural antioxidant that were Present in *Cassia fistula* Linn. are responsible for inhibiting or Preventing the deleterious consequences of Oxidative stress. Thus it is clear from graph that Alcoholic extract of the barks of this Plant exhibited best antioxidant activity or potential than the other extracts when Compare to Standard drug ascorbic acid too. It showed 81.19% inhibition at max 30µg/ml Concentration IC 50 value was obtained as approx 15-16 µg/ml.

CONCLUSION

In the Present study, Bark of *Cassia fistula* Linn. Were subjected to extraction using 95% v/v alcohol, chloroform, Petroleum ether and water. Then, these extracts were subjected for Phytochemical screening and Qualitative TLC analysis.

Results revealed that barks contained saponin glycosides, fats and volatile oil. Aqueous extract contains Saponin glycosides and Volatile oil. The AlcE showed the Presence of saponin glycoside, CE contain volatile oil and PEE, showed the Presence of fats and oils.

The Physicochemical evaluation of *Cassia fistula* has shown

1. % Loss of drying = 17.2%.

2. % Total ash value = 7.5%.
3. % Acid Insoluble ash value = 1%.
4. % Water soluble ash value = 0.025%.

The following R_f values were obtained with respect to various combinations of solvents.

1. R_f Values = 0.90,0.22,0.18, 0.98,0.81, 0.96, 0.98, 0.97, 0.86, 0.96, 0.92, 0.93.

The extractive values were found as

1. Alcoholic soluble extractive value = 20%.
2. Water soluble extractive value = 30%.

The antioxidant activity *Cassia fistula* can be determined accurately, conveniently and rapidly using DPPH scavenging assay method. It is also clear that Alcoholic extract of the Parts i.e barks of this Plant exhibited best antioxidant Potential then the other extract when compare to standard drug Ascorbic acid. It showed 81.19% inhibition at max 30µg/ml. Concentration. IC 50 value was obtained and found to be as approx 15-16µg/ml.

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