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Physico-chemical characterstics, proximate analysis and total phenolic content of *Cassia fistula* bark

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ABSTRACT

To investigate the medicinal value of *Cassia fistula* Linn., bark nutritive value, proximate analysis, total phenols concentration and various characteristics has been determined. The bark of C*assia fistula* contain moisture content 7.760±0.49 which is under in acceptable limit, total carbohydrate content found 72.476±0.48, it also having good content of crude fiber and crude protein as 32.263±0.749 and 4.889±0.174, bark of *Cassia fistula* is great source of energy for plant as it contain high quantity of nutritive value (332.654±3.19 Kcal/100 g). Total ash found 12.297±0.01 in which 6.688±0.04 is acid insoluble ash while 0.716±0.02 is water soluble ash. Alcohol soluble extractive value 10.036±0.29 shows that most of the phytoconstituent are extracted by alcohol. The extraction of bark is performed by various solvent in soxhlet extractor. High acid value obtained in acetone and ethanol extract as 560.728±0.45 and 448.207±1.87 respectively which is responsible for high Saponification value 618.694±1.54, 661.211±1.05. Excellence iodine value 116.389±1.15 is obtained in diethyl ether extract. The concentration of total phenols is calculated by Folin-Ciocalteu method. Diethyl ether shows excellence concentration 101.585±0.07μg/ml of total phenols while acetone and ethanol extract having appreciable total Phenolic concentration (72.228±0.07μg/ml and 53.085±0.07μg/ml).   © 2013 Trade Science Inc. - INDIA

INTRODUCTION

It is concerned here that Indian medicinal plants are considered as a vast source of several pharmacologically activities and compounds that are commonly used as home remedies against multiple ailments[1]. Proximate and nutrient analysis of plants plays a crucial role in assessing their nutritional significance[2]. The previously research shown that phenolic compounds such as catechin and quercetin were very efficient in stabilizing phospholipid bilayers against peroxidation induced by reactive oxygen species (ROS)[3,4]. In this regard, one of the important medicinal plant is *Cassia fistula* which is fast-growing, medium-sized, deciduous tree about 9 meters in height belongs to Ceasalpiniacae (Fabaceae) family commonly known as Amultas in hindi and Indian Laburnam[5], (Golden Shower) in english. Traditionally, it is used in the treatment of hematemesis, pruritis, intestinal disorders, leucoderma, diabetes, and as antipyretic, analgesic and laxative[6,7], various parts of *C. fistula* plant are known to be an important source of secondary metabolites, notably phenolic com-

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pounds[8]. Hence, the present work is aimed to analysis the physico-chemical properties of various extracts and determined their total phenols concentration. The present work also assembles ash analysis as physical evaluation, proximate analysis and nutritive value on the mature bark of *C. fistula* from Haridwar (Uttarakhand), India.

MATERIAL AND METHODS

Collection and identification of plant material

Mature bark of *C. fistula* was collected from Haridwar region in month of February 2011. The authentication of plant is carried out by the Botanical Survey of India, (BSI), Dehradun with Acc. No. 113637 and a voucher specimen has been deposited in medicinal plants herbarium in Department of Chemistry, Gurukula Kangri Vishwavidyalaya, Haridwar under the registry No. 22/15. The mature bark was in shade dried and grinded in to powder form in pestle mortar and stored in polybag till further uses.

Chemical and reagents

The various solvent used for extraction were (C.D.H. Pvt. Ltd., New Delhi). To determination of total phenolic content, Folin-Ciocalteu reagent (Merck Pvt. Ltd, India), Gallic acid (Loba chem.) and sodium carbonate (CDH P. Ltd., New Delhi) were used. Distilled water was used to prepare the solution and other experimental procedure.

Instrumentation

The extraction was carried out by 800 ml soxhlet extractor (J.S.G.W.) and concentrated in rotary vacuum evaporator (Labco). Kjeldal flask (J.S.G.W.) for total nitrogen and other glassware (Borosillicate) for various procedures were used. The absorbance was measured by Shimadzu U.V. 2400 Spectrophotometer (Shimadzu, Japan).

Extraction of plant material

200 g of crushed bark material of *C. fistula* were extracted sequentially and successively in 1.5 litre of solvent with increasing order of polarity i.e. petroleum ether (40-60°C), benzene, chloroform, diethyl ether, acetone, ethanol and finally with distilled water. All extracts were filtered through Whatmann filter paper No. 42 and all extracts were concentrated at reduced pres-

sure using vacuum rotary evaporator. After concentration, solvent free extracts were sealed in bottle and kept in refrigerator.

Physico-chemical analysis

The various extracts of *C. fistula* were investigate for their different physical properties i.e. solubility, colour, p^H value, refractive index and optical rotation of extract and different chemical parameter i.e. acid value, iodine value, saponification value and ester value. All parameters were according to standard method of Indian pharmacopeia[9].

Physical evaluation

The shade dried mature bark of *C. fistula* was subjected to ash value and extractive value determination. Total ash, acid insoluble ash, acid soluble ash, water soluble ash and sulphated ash were determined as ash value parameter for physical evaluation whereas alcohol soluble extractive value and water soluble extractive value were determined for extractive value parameter. These all parameters were subjected to quantitative test analysis using standard method[10].

Proximate analysis and nutritive value

The parameters of proximate analysis are: percentage of moisture content, total nitrogen content, crude protein, crude fat, crude fiber, total carbohydrate content and available carbohydrate content. Moisture content was estimated directly by the weight difference of *C. fistula* bark on drying 105ºC on oven. Total nitrogen determined by Kjeldal method while crude protein were calculated by following formula as (Crude Protein (CP) % = %N x 6.25). Crude fat was evaluated by soxhlet extraction of plant material by a non polar solvent as petroleum ether and after defatting, the fat free material was used for crude fiber estimation. Total carbohydrate content was calculated by standard formula as:-

Total carbohydrate content = 100 – (% of ash + % of moisture + % of crude fat + % of crude protein).

By subtracting percentage of crude fiber from the total carbohydrate content, available carbohydrate content in plant material is evaluated i.e.

Available Carbohydrate content = (Total Carbohydrate Content - % of Crude fiber)

Now the Nutritional value of *C. fistula* bark (Kcal/100g) was determined by applying following formula.

Nutritative value (Kcal/100 gm) = (4 X % Protein) + (9 X % Crude Fat) + (4 X % Carbohydrate)

All these processes were adopted by AOAC and other standard methods[11-17].

Total phenolic content

The total phenol contents in various extract of *C. fistula* were measured spectrophotometrically with Folin–Ciocalteu reagent followed the protocol in food and analytical chemistry[18]. Gallic acid solution was used as a standard solution for the determination of total phenols. The concentration of total phenols in each extract of *C. fistula* was measured by the calibration curve of standard Gallic acid solution of various dilutions. Procedurally take 1 ml of standard Gallic acid solution of various dilutions and add 38.5 ml distilled water with 2.5 ml of Folin–Ciocalteu reagent. These content mix properly and stand for incubation at room temperature for 8 minutes. Incubation time should not be more than 8 minute. After incubation, add 8 ml of Na_2CO_3 solution and this reaction mixture is stand for two hours. When reaction has been completed take 2 ml of reaction mixture sample in quartz cuvette and measured absorbance at 765 nm. The same process adopted for extract sample. The blank determination was also measured by taking 1 ml of distilled water.

RESULT AND DISCUSSION

Successively soxhlet extraction was carried out for *C. fistula* mature bark with various solvents as petroleum ether, benzene, diethyl ether, chloroform, acetone, ethanol and distilled water gives yield and consistency after concentration of extract. TABLE 1 show the % yield (w/w) of different extract of bark of *C. fistula.*

TABLE 1 : The percentage yield, colour and physical state of vaious extract

Extract	Weight of sample(g)	Weight of extract(g)	% Yield (w/w)	Colour	Consistency
PE	200	0.9	0.45	Yellowish	Waxy
BZ	200	1.1	0.55	Yellowish	Waxy
DE	200	1.1	0.55	Yellowish	Semi solid
CL	200	1.2	0.60	Light green	Waxy
AT	200	13.2	6.60	Reddish brown	Crystalline
ET	200	8.0	4.00	Reddish brown	Crystalline
AQ	200	6.5	3.25	Black	Crystalline

PE = Petroleum ether, BZ = Benzene, DE = Diethyl ether, CL = Chloroform, AT = Acetone, ET = Ethanol, AQ = Aquas

TABLE 2 : Physico-chemical analysis for the various extract of *C. fistula* bark

Parameter	Sub Parameter	Observed value						
		PE	BZ	DE	CL	AT	ET	AQ
Physical Parameter	Colour	Yellowish	Yellowish	Yellowish	Light green	Reddishbrown	Reddishbrown	Black
	Odour	Charcterstic	Charcterstic	Charcterstic	Charcterstic	Sweet	Sweet	Salty
	R.I.	1.330±0	1.333±0	1.333±0	1.331±0	1.328±0	1.331±0	1.332±0
	p^H	4.7±0	4.8±0	4.3±0	4.0±0	4.2±0	4.4±0	5.2±0
	Opt. rot.	+3.5±0	+2.5±0	+0.8±0	+2.3±0	+2.6±0	+2.0±0	+3.7±0
	Solubility	Chloroform	Chloroform	Chloroform	Chloroform	Ethanol	Methanol	DistilledWater
Chemical Parameter	Acid value	64.786 ±0.14	46.610±0.34	37.605±0.458	53.197±0.39	560.728±0.45	448.207±1.87	15.446±0.97
	Ester value	210.860±0.62	41.941±1.98	247.019±1.22	207.390±1.22	57.966 ±1.16	213.004±1.48	74.643±2.64
	Iodine value	38.368±1.41	37.413±0.69	116.389±1.15	21.227±1.23	86.684 ±2.05	46.245±1.01	12.531±1.326
	Sap. value	275.646±0.50	88.551±1.67	284.624±1.25	260.588±1.60	618.694±1.54	661.211±1.05	90.109±1.76

* The results are shown in mean ± S.D.
Opt. rot. =Optical Rotation, R.I. = Refractive Index, Sap. Value = Saponification Value PE = Petroleum ether, BZ = Benzene, DE = Diethyl ether, CL = Chloroform, AT = Acetone, ET = Ethanol, AQ = Aquas

Physico-chemical evaluation of *C. fistula* bark extracts have been done in which colour, odour, refractive index, p^H were analyzed under physical parameter while in chemical parameter acid value, saponification value, ester value and iodine value, shown in TABLE 2. The colour of petroleum ether, benzene, diethyl ether extracts are yellow, chloroform extract's colour is light green while reddish brown colour are found in acetone and ethanol extracts. All extracts have characteristic smell while acetone and ethanol extract have characteristic sweet smell. Most of the extracts are soluble in chloroform. P^H values justify the acidic nature of extracts. Optical rotation and refractive index were measured at 30ºC. Acetone extract has highest acid value

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while maximum saponification value is found in ethanol. Diethyl ether extract have highest ester value and iodine value. Due to higher iodine value in diethyl ether extract shows high degree of unsaturated hydrocarbons present in this extract.

The various calculated value of physical evaluation of *C. fistula* bark is present in TABLE 3. It shows the percentage of ash content and extractive value. The extractive values were analyzed with two solvent i.e. alcohol and water whose extractive values are 10.036% and 5.752% respectively. It clears that alcohol is more suitable solvent for extraction. Percentage of total ash value justifies the percentage of inorganic matter present in plant sample[19] and it is a diagnostic purity index and represents the physiological ash and non-physiological ash[20].

TABLE 3 : Results of physical evaluation of *C. fistula* bark

Parameter	Sub- Parameter	Value (%)
Ash Value	Total Ash	12.297±0.012
	Acid Insoluble Ash	6.688±0.042
	Acid Soluble Ash	5.610±0.036
	Water Soluble Ash	0.716±0.027
	Sulphated Ash	12.452±0.297
Extractive value	Alcohol Soluble	10.036±0.294
	Water Soluble	5.752±0.587

* The results are shown in mean ± S.D

The quantitative determination of total phenols in various extracts of *C. fistula* bark is presented on TABLE 5. The concentrations of Phenolic content in various extract were determined by Folin - Ciocalteu method which is shown in terms of mg GAE/g dw. Gallic acid was used as a standard whose Linear equation is $y = 0.0014x + 0.0278$ shown in Figure 1. The total phenolic content in various extracts is: petroleum ether 19.085, benzene-23.800, diethyl ether 101.585, chloroform 36.657, acetone 72.228, ethanol 53.085 and aquas extract 19.085±0.07mgGAE/g dw. It shows the diethyl ether extract is great source of total phenols while appreciable amount of total phenols are also present in acetone and ethanol extract. Phenolic compounds are a class of antioxidant agents which acts as free radical terminators[25], i.e. diethyl ether extract having excellence antioxidant activity while acetone and ethanol extract act as a good free radical terminators and also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores[26].

TABLE 4 : Proximate parameter for *C. fistula* bark

Parameter	Value (%)
Moisture Content or Loss on Drying (LOD)	7.760 ± 0.496
Total Nitrogen Content	0.782 ± 0.027
Crude Protein	4.889 ± 0.174
Crude Fat	2.577 ± 0.361
Crude Fiber	32.263 ± 0.749
Total Carbohydrate Content	72.476 ± 0.483
Available Carbohydrate Content	40.213 ± 0.915

Nutritive value (Kcal/ 100 g) = 332.654±3.190
* The results are shown in mean ± S.D

TABLE 5 : Concentration of total phenolic content in various extract of *C. fistula*

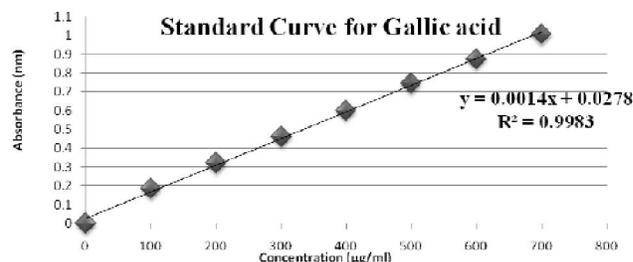| ABSORBANCE	CONCENTRATION (µg/ml)
PE	19.085 ± 0.07
BZ	23.800 ± 0.07
DE	101.585 ± 0.07
CL	36.657 ± 0.07
AT	72.228 ± 0.07
ET	53.085 ± 0.07
AQ	19.085 ± 0.07

Figure 1 : Standard curve for standard Calibration Gallic acid

STATICAL ANALYSIS

The analysis results processed in triplicate readings and their mean value, standard deviation were calculated by sigma state software.

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