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Preliminary investigation and antimicrobial screening of successive extracts of phytoconstituents from *Cassia fistula* of Haridwar region, India

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Abstract

***Cassia fistula* belongs to family leguminosae. It is a medium sized tree and its different parts are used in Ayurvedic medicine as well as in home remedies for common ailments. The plant is easily available in Haridwar region. The phytoconstituents of a same plant vary from region to region. In the present study bark of *Cassia fistula* is used. The material was collected (in Haridwar region, India), dried in shade; powdered and extracted successively with different solvents in an increasing order of polarity. Phytochemical investigation was performed using different identification tests. The different extracts were also screened for antimicrobial activity. For which both Gram positive and Gram negative bacterial strain were selected. Antimicrobial test was performed by agar well diffusion method. All the tests were performed in a triplicate. The different phytoconstituents present in the bark extract are responsible for such an appreciable activity against selected pathogens.**

Keywords: *Cassia fistula, successive extraction, phytochemical investigation and antimicrobial screening*

Introduction

Infectious disease caused by bacteria, viruses, fungi and parasites are still a major threat to public health, despite the tremendous progress in human medicine (Cosa *et al*. 2006). *Cassia fistula* L., Caesalpiniaceae (Leguminosae), a semi-wild Indian Labernum (also known as the Golden Shower), is distributed in various countries including Asia, South Africa, Mexico, China, West Indies, East Africa and Brazil. It is an ornamental tree with beautiful bunches of yellow flowers (Duraipandiyan and Ignacimuthu, 2007). As there are many climatic condition in India so as climatic condition varied, percentage of phytoconstituents also varied in same plant. Uttarakhand state of India is popular for its climatic diversity. A number of medicinal plants are cultivated in Uttarkhand region. *Cassia fistula* is one of the medicinally valued plant belonging to Caesalpiniaceae family habited also in Haridwar region. *Cassia fistula* is a moderate size deciduous tree, leaflets 8 to 12 pair, flowers yellow, long drooping racemes, pod cylindrical and pulpy, seeds light brown, hard shiny (Stephen). A rare study is done on the mature bark mainly by the exhaustive and sequentially technique. Also no work is reported specially for Haridwar region. So, this work present the different phyto constituents extracted successively by different solvent on increasing order of polarity and their antimicrobial activity specially from Haridwar region .

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Material and Methods

Plant Material:- Mature bark of *Cassia fistula* were collected from Haridwar locality in month of February 2011. The taxonomic identity of plant was confirmed by the Botanical Survey of India, (BSI), 192 Kaulagarh road, Dehradun. Two set of plant herbarium is deposited in Botanical Survey of India, Northern regional centre, Dehradun (BSD) in which one set of authenticated voucher specimens **Acc. No. – 113637** is received and deposit in the department of chemistry, Gurukul Kangri Vishwavidyalya, Haridwar, Uttarkhand. The mature bark was shade dried and grinded in to powder form in pestle mortar and stored in polybag until further uses.

Extraction of plant material :- 200 g of crushed bark of *Cassia fistula* were extracted sequentially and successively with solvent in increasing order of polarity as petroleum ether (40-60°C), Benzene, Acetone are concentrated at reduced pressure using

rotary vacuum evaporator. After concentration, solvent free extracts and were sealed in bottle and kept in refrigerator for phytochemical and anti-microbial screening.

Preliminary phytochemical screening:- The phytoconstituents present in different extract were analysed by using standard qualitative method (Kokate *et al*. 2006 and Harborne, 1984).

Antimicrobial study

Used bacterial strain: **-** Four bacterial strain were used in this work in which two were gram negative and two were gram positive human pathogenic bacterial strains. The test microorganisms used for present work are *Escherichia coli (***ATCC 433),** *Bacillus cereus* **(ATCC 11778),** *Bacillus licheniformis* **(ATCC 1483)** and *Salmonella typhi* **(ATCC 733)**. All the stock cultures in pure form were collected from S.G.R.R.I.T.S department of Life Sciences, Dehradun. All the bacterial strain were identified by standard methods.

Bacterial culture media and inoculums: **-** Muller Hinton Agar (Hi-Media Pvt. Ltd., Bombay, India) is used to grow the culture of these test bacteria's. 30 g of Muller Hinton Agar was weighed out and dissolved in 800ml of distilled water in a conical flask and pH of the solution is maintained in between 4.5 to 5.5. This flask is kept in autoclave at 121°C for 15 minute. Muller Hinton Agar was poured on sterilized four petriplates and spreaded out. All these process were carried out in a laminar air flow. All the plates were kept in B.O.D. incubator at 37°C for culture growth for 24 hours. Culture is diluted in sterile normal saline solution and the turbidity of the suspension is adjusted equivalent to a 0.5 McFarland standard by adding more bacterial strain, so as to obtain the cell suspension between 10^5 to10^8 CFU/ml.

Preparation of test extract for microbial screening: - The solvent free extract of *Cassia fistula* bark is dissolved in 0.5 ml of sterilized and filtered DMSO (filtered with whatman filter of pore size 0.45 micron) to prepare a test solution of extract of desired concentration for microbial screening.

Antimicrobial assay: **-** The determination of antibacterial screening of different extract of *Cassia fistula* bark is carried out by agar well diffusion technique (Adeniyi *et al*.1996). Ofloxacin drug is used to as a standard drug.

Results and Discussion

***Yield of different extracts*:-** After complete extraction the extract is concentrated which gave yield and consistency of different extract. Table No. 1 shows the % yield (w/w) of different extract of bark of *Cassia fistula.* The percentage yield is in small amount in Petroleum extract i.e. small concentrations of phytoconstituents are present in petroleum ether extract. In the same way appreciable amount of phytoconstituents are present in acetone extract which shows higher value.

Table No. 1:- The percentage yield, colour and physical state of concentrated different extract of *Cassia fistula* bark.

Extracts	Weight of sample (gm)	Weight of extract (gm)	w/w % yield	Colour	Consistency
Petroleum ether	200	0.9	0.45	Yellowish	Waxy
Benzene	200	1.1	0.55	Yellowish	Waxy
Acetone	200	13.2	6.6	Reddish brown	Crystalline

Preliminary Phytochemical Screening: **-** The preliminary phytochemical investigation is carried out by their different test or specific test in each extract of *Cassia fistula* bark which show the bioactive secondary metabolic constituent as in Table no. 2. Acetone extract gave excellent result of different phytoconstituents while petroleum ether and benzene extract show comparatively moderate result. Steroid, carbohydrate, proteins, phenolic compound and tannin, cardiac glycosides are present in appreciable amount in acetone extract.Inulin are present in all extract while aleurone grains, amino acid, flavanol glycosides, gums and mucilage, naphthoquinones are absent.

Antimicrobial Screening

In this study microbial screening was also performed by the different extract of *Cassia fistula* bark against selected microorganism in which two Gram negative and two Gram positive human pathogenic microorganism were used to test its resistance activity.The screening performs excellent results against these bacterial strains.The zone of inhibition justify that this plant exhibited antimicrobial activity. The activity in terms of zone of inhibition is noted from petriplates and are presented in Table No. 3. The acetone extract exhibits the maximum zone of inhibition against *Bacillus cereus.*

Petroleum ether and Benzene extracts are weekly effective against bacterial strain. Fig No. 1 shows graphical representation of zone of inhibition of different extract of *Cassia fistula* bark against the selected human pathogens.

Table No. : - 2 The phytochemical tests are performed for the Petroleum ether, Benzene and Acetone extract.

Phytoconstituents and Test performed			**Extracts**		
			Petroleum ether	**Benzene**	**Acetone**
1. Aleurone grains			-	-	-
2. Alkaloids	*Mayer's Test*		-	-	-
	Wagner's Test		-	-	-
	Hager's Test		+	+	+
	Tannic acid Test		+	+	++
3. Carbohydrate	*Molisch's Test*		+	+	+
	Fehling's Test		-	+	+++
	Benedict's Test		-	-	+++
	Selivanoff's Test		-	-	-
4. Glycosides	***Anthraquinone glycosides***	*Borntrager's Test*	-	-	-
		Test for Hydroxy-anthraquinones	-	-	+
	Cardiac glycosides	*Keller-Killiani Test*	-	-	-
		Legal's Test	-	-	+++
		Baljet's Test	-	-	_
	Saponin glycosides	*Froth formation Test*	-	-	+
	Flavanol glycosides	*Mg and HCl reduction*	-	-	-
5. Inulin			+	+	++

6. Protein	*Heat Test*	-	-	-
	Biuret Test	-	-	+
	Xanthoproteic Test	-	-	+++
7. Amino Acid	*Ninhydrin Test*	-	-	-
8. Steroids and Triterpenoids	*Salkowski Test*	-	-	+++(s)
9. Fixed oils and Fats	*Spot Test*	+	+	-
	Saponification Test	-	-	-
10. Flavonoids	*Shinoda Test*	-	-	++
	Alkaline reagent Test	-	-	++
	Zinc hydrochloride Test	-	-	-
11. Phenolic compounds and Tannins	*Lead Acetate Test*	-	-	+++
	Ferric chloride Test	-	-	+++
	Test for Catechin	-	-	-
	Test for Chlorogenic acid	-	-	-
12. Gums and Mucilage		-	-	-
13. Naphthoquinone	*Juglone Test*	-	-	-
	Dam-Karrer Test	-	-	-

(s) = Steroids, (+++) = Appreciable amount, (++) = Moderate amount, (-) = Absence

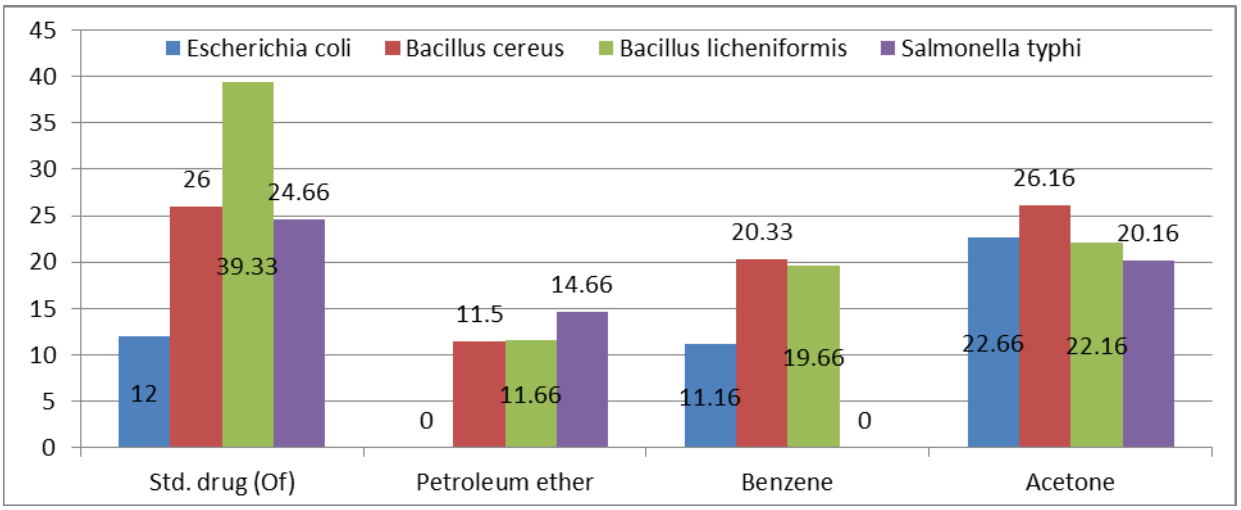
Table No. 3:- Antimicrobial investigation of different extract of *Cassia fistula* bark against selected microorganism. All the values are mean zone of inhibition ± SD

Bacterial strain	**Zone of inhibition in mm. (Mean ± SD)**			
	Std. drug (Of)	Petroleum ether	Benzene	Acetone
Escherichia coli	12 ± 1.00	-	11.16 ±1.04	22.66 ± 1.52
Bacillus cereus	26 ± 1.00	11.5 ± 1.32	20.3 ± 1.52	26.16 ± 0.76
Bacillus licheniformis	39.33 ± 1.15	11.66 ± 1.52	19.66 ± 1.52	22.16 ± 1.25
Salmonella typhi	24.66 ± 1.52	14.66 ± 2.51	-	20.16 ± 1.75

(-) = no zone of inhibition, Std. drug (Of) = Standard control drug Ofloxacin

A number of allopathic drugs are used to prevent the infection against human and animal pathogenic bacteria which are also having their side effects. The demand of herbal medicine shows that plant medicines are the part of human life which have no side effects. As Indian rural population are completely depending upon herbal medicine for their primary health care. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world population. Over the years, the World Health Organization advocated that countries should interact with traditional medicine with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments of both microbial and non-microbial origins (World Health Organization, 1978).

Fig. no.: - 1 Graphical representation of extracts against selected bacterial strains



In the present study the *Cassia fistula* bark against acetone extract showed excellent zone of inhibition for tested bacteria. The microbial activity of the *Cassia fistula* was due to the presence of various secondary metabolites (Nayan, 2011). Table No. 2 shows that a number of secondary metabolites are present in acetone extract as carbohydrate, cardiac glycosides, Inulin, protein, steroids, flavonoids, phenolic compounds and tannins which are responsible for their microbial activity. In recent years there has been a resurgence of scientific interest in the use of medicinal plants for the development of new phannacotherapeutic agents against different species of microorganisms including the resistance organisms (Hatano, 1999 and Palombo, 2002). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Runyoro, 2006 and Shahidi, 2004). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides etc, which have been found *in vitro* to have antimicrobial properties (Dahanukar, 2000 and Cowan, 1999).The above study confirms that a number of phytoconstituents in appreciable amount are present in acetone extract which may be responsible for their antimicrobial activity. Most of the phytoconstituents are hydrophilic in promising extract. Finally further researches on plant derived antimicrobials are needed so as to determine the identity of that particular compound in this plant by different technique and also to determine their full spectrum.

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