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PRELIMINARY PHYTOCHEMICAL, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF DIFFERENT EXTRACTS OF *CASSIA TORA* AND *TRICHODESMA INDICUM*

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Abstract

The present study deals with the phytochemical, antioxidant and anti microbial activities of two medicinal plants, namely, *Cassia tora* and *Trichodesma indicum*. Choloroform, ethyl acetate, ethanol and hydroethanolic solvents were used to extract the phytochemicals present in these two plants. The extracts were studied for the presence of different phytochemicals.

Further, the antioxidant and antimicrobial properties of these extracts were studies following standard procedures. It was found that tannins, steroids and triterpinoids were present in all the extracts of both the plants whereas flavonoids, anthraquinones, cardiac glycosides, proteins, amino acids were absent in all the extracts of both plants. *Cassia tora* indicated more antibacterial and antifungal activities as compared to *Trichodesma indicum*. The antioxidant property of *Trichodesma indicum* was interestingly more as compared to *Cassia tora*. Further study on these parameters will be useful to develop medicines with these plant extracts to avoid the use of chemicals as drugs.

Key words: Antibacterial, Antibacterial, Antifungal, *Cassia tora,* Phytochemical, *Trichodesma indicum,*

1. Introduction

Plants are the richest resource of drugs. Traditional systems of medicine, modern medicines, neutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs are directly or indirectly dependent on plants [1]. The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is founds in "Rigveda", which is said to have been written between 4500 -1600 B.C. and is supposed to be the oldest repository of human knowledge.

It is Ayurveda, the foundation of medicinal science of Hindu culture, which deals with specific properties of drugs and various aspects of science of life and the art of healing [2]. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country.

Herbal medicine is still the mainstay of about 75 - 80% of the whole population, and the major part of traditional therapy involves the use of plant extract and their active constituents [3]. Among the 7,000 species of medicinal plants recognized all over the world, more than 900 types of precious medicinal plants are found in India. Unfortunately, only few of them are used for their medicinal value. About 1500 plants are systematically used in indigenous system of medicine, like Ayurveda, Unani and Siddha.

However, the ethno pharmacologists, botanists, microbiologists and natural product chemists world over today, is constantly still in search of medicinal efficacy of plants and their phyto-chemicals, since the reported data so far available on plants are comparatively meager as compared to the vast number of plant population. The drugs which are already in use to treat infectious diseases is of concern because, drug safety remains an enormous global issue. It was estimated that 2.22 million hospitalized patients had serious Adverse Drug Reactions (ADR) and 106,000 died in a single year in the USA.

The Herbal and natural products have been used in folk medicine for centuries throughout the world, but there are relatively lower incidences of adverse reactions to plant preparations compared to modern conventional pharmaceuticals. This, coupled with their reduced cost, is encouraging for both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs [4].

Siddha and Ayurvedic systems of medicine are the oldest systems of medicine in India. Herbal medicines are being used by about 80% of the world population mostly in the developing countries for primary health care. These medicines have stood the test of time for their safety, efficacy, cultural acceptability and lesser side effects. Indian medicinal plants and their derivatives have been an invaluable source of therapy due to their antibacterial, antihelmintic, anti ulcer, anti-inflammatory, anticancer, antioxidant and anti-inflammatory and many other medicinal properties [5].

Phytochemicals are, in the strictest sense of the word, chemicals produced by plants. Plants generally contain phyto-constituents like anthraglycosides, phlobatanin, tannins, reducing sugars, flavonoids, alkaloids, saponins, coumarins,

phenols, carboxylic acids, terpenes, tannins, caffeine, etc. These phyto-constituents confer specific characteristics and properties to plants. The constituents of plants are known for their medicinal value to treat various ailments since time immemorial. The discovery, development and use of modern medicines have a deep rooted connection with the age old practice of folk and traditional medicinal background of the natives [6]. In recent times focus on plant research has increased around the world and a large body of evidence has been collected to show the immense potential of medicinal plants used in various traditional systems of medicine.[6] The practice of native medicine is the synthesis of therapeutic experience of generations of practicing physicians. Herbal medicines have played a great role in the cure of many diseases in the history of mankind. Thus the ancient wisdom has been the basis of modern medicine and will remain so. Therefore, the analysis of these constituents in plants would help in determining various biological activities of plant products. The extraction methods and use of the phyto-chemicals as important sources of medicine and therapeutic uses are well documented [7, 8, 9].

In the present study we have analyzed two medicinal plants, namely, *Cassia tora* and *Trichodesma indicum*, for their phytochemicals, antibacterial, antifungal and antioxidant activities of the crude extracts using four solvents, namely, chloroform, ethyl acetate, ethanol and hydroethanol(20% Ethanol: 80% Distilled water). These two plants, although belong to different texa have many ethno medicinal applications which are common. Although a lot of work has been done on these two plants the present study envisages in understanding the similarities and differences among these two plants by using a few parameters.

Cassia tora

Figure 1. Twig of *Cassia tora*

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Fabale

Family: Fabaceae

Subfamily: Caesalpinioideae

Tribe: Cassieae

Sub-tribe: Cassiinae

Genus: *Cassia*

Species: *Cassia tora*

Habitat: Grows in warm moist soil throughout tropical parts of India.

Distribution

This plant is available in India, Iran and Mauritius.

Description

An annual foetid herb, with a height of 30 to 90 cm, *Cassia tora* is mainly found in the states of Uttar Pradesh and Madhya Pradesh, in India. It has pinnate leaves, which are about 10 cm long. Each leaf has three pairs of leaflets that are opposite, ovate, oblong and oblique at the base. The yellow-colored flowers are bearded in the axel of the leaves.

The flowers comprises of five petals, each about half inch in diameter.

The seeds of *Cassia tora* are rhombohedral and brown in colour, about 30 to 50 in number. The plant bears flowers in the rainy season and fruits in the winter.

Plant Chemicals

The following phytoconstiuents are reported to be present in *Cassia tora* plant, (+)- rhein, aloe-emodin, chrysophanol, 7% resins, cathatrine, calcium, iron, phosphorus, 1,3,5-trihydroxy-6-7-dimethoxy-2-methylanthroquinone, beta-sitosterol, naptho-alpha-pyrone-toralactune, chrysophanol, physcion, emodin, rubrofusarin, cchrysophonic acid-9-anthrone, tricontan-1-0l, stigmasterol, b-sitosteral-b-D-glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin, isoquercitrin.

The medicinal values of *Cassia tora* and *Trichodesma indicum* is well documented by various researchers.

Das *et al*, 2011 and Pawar and D'mello, 2011 have presented an overview of pharmacological features of *Cassia tora* [10, 11]. The leaf extract of *Cassia tora* plant is reported to have cardio protective activity in myocardial injury [12]. The

antibacterial activity of leaf extracts on various human pathogens were reported by Chavan *et al*, 2011, Ropashree *et al*, 2010, Sharma *et al*, 2010, Rao *et al*, 2012, Gill *et al*, 2011[13, 14, 15,16, 17] . The antioxidant properties of topical cream prepared by *Cassia tora* leaves is reported by Gupta *et al*, 2012 [18]. The in vitro anthelmintic activity of *Cassia tora* was reported by Kawade and Manisha, 2013 [19]. The antifungal activity of leaf extract was reported by Mukherjee *et al*, 1996 [20]. The antiarthritic activity of *Cassia tora* plant parts was reported by Balekar *et al*, 2013 [21].The antidiabetic activity of *Cassia tora* leaf was reported by Chaurasia *et al*, 2011. Rejiya *et al*, 2009 have reported the anticancer properties of *Cassia tora* leaves [22, 23]. Tamhane *et al*, 2012, have reported the antihistaminic activity of this plant [24]. Ethnobotanically this plant has been used for many other medicinal aspects. *Cassia tora* is used as a coffee substitute and has a maturing and anodyne action. It is very useful in treating skin diseases like ringworm and itching or body scratch and psoriasis. Decoction of the fruit of *Cassia tora* is used in the treatment of fever. Since it is considered to be a kapha and vata dosha suppressant, it acts as a nerve tonic. It is consumed in worm infestation. *Cassia tora* acts as a liver stimulant, mild laxative and heart tonic. The herb helps the body in maintaining the normal level of cholesterol. *Cassia tora* proves worthwhile in treating piles and haemorrhoids as well as relieving the pain caused on excretion. Its powder proves useful in combating indigestion, toning up heart muscles and purifying blood. The juice extracted from its leaves is used in case of skin ailments, rashes and allergies. It is also used as an antidote in case of various poisonings.

Trichodesma indicum

Kingdom: Plantae

Phylum: Magnoliophyta

Class: Magnoiotate

Order: Lamiales

Family: Boragenaceae

Genus: *Trichodesma*

Species: *Trichodesma indicum*

Habitat: This plant is available in India, Iran and Mauritius.

Figure 2. Twig of *Trichdesma indicum*.

Description

An erect, spreading, branched, annual herb, about 50 cm in height. Leaves are stalkless, opposite, lanceolate, 2 to 8 cm long, pointed at the tip, and heart-shaped at the base; the upper surfaces clothed with stiff hairs arising from circular tubercles, the lower surfaces less densely villous. Flowers occur singly in the axils of the leaves. Calyx is green, hairy,

and 1 to 1.3 cm long, with pointed lobes. Corolla is pale blue, with the limb about 1.5 cm in diameter, and the lobes pointed. Fruit is ellipsoid, and enclosed by the calyx. Nutlets are about 5 mm long, and rough on the inner surface.

Plant Chemicals

The plant contains phytochemicals like, steroidal, ß-sitosterol and phenolics, catechin and gallic acid. It Yields hexacosane, ethyl hexacosanoate and 21, 24-hexacosadienoic acid ethyl esters. Seed oil yield oleic, linoleic, palmitic, stearic, and linolenic acid. In Indian traditional medicine, the decoction of roots used for diarrhoea, dysentery and fever. In Deccan, the plant is used as emollient poultice. In Chutia Nagpur, roots are crushed and made into a paste, and applied externally to swollen joints, inflammations and superficial skin injuries. Used for arthralgias, inflammations, dyspepsia, diarrhoea, dysentery, dysmenorrhea. In Tamil Nadu, Southern India, root decoction taken internally to treat bloody dysentery. *Trichodesma indicum* is acrid and bitter tasting and considered to be thermogenic, emollient, alexeteric, anodyne, anti-inflammatory, carminative, constipating, diuretic, depurative, ophthalmic, febrifuge and pectoral. Flowers are considered sudorific and pectoral. Leaves considered depurative. The roots, leaves and flowers are used for medicinal purposes. Leaves and flowers are edible. In the Philippines, *Trichodesma zeylanicum* leaves and roots are used as remedy for snake bites and also used as diuretic. Cold infusion of leaves considered depurative. Crushed roots, in decoction or infusion, used for dysentery in children. Study of methanol extract of the whole plant of *Trichodesma indicum* on sulphur dioxide-induced cough reflex in Swiss albino mice showed significant inhibition of cough frequency in all tested doses compared with the untreated control group, in an effect comparable to codeine phosphate [25]. Extract study showed significant inhibition of castor oil-induced diarrhoea and decreased propulsion of charcoal meal through the GI tract. There was also reduction of castor oil-induced enteropooling. Results support the use of the herbal remedy as a nonspecific treatment for diarrhoea in folk medicine [26]. Effect of *Trichodesma indicum* extract on cough reflex induced by sulphur dioxide in mice was studied by Srikanth *et al* 2002 [27]. The chloroform extract of *T. indicum* root exhibited significant anti-inflammatory activity in acute and chronic inflammatory models [28]. Study of some Pakistani medicinal plants showed the extract of *T. indicum* caused reduction in spontaneous and acetylcholine-induced contractions, with 78% inhibition of intestinal contractions and good lipooxygenase inhibitory activity [28]. Study evaluated antimicrobial activity of *Trichodesma indicum* and *T. sedgwickianum* against bacteria and fungi. The ethanol extract of both species was more active against gram positive bacteria, *S. aureus* and *B. subtilis* while the aqueous extract showed strong

inhibitory activity against gram negative bacteria like *E. coli* [29, 30]. Analgesic and antipyretic activity of ethanolic root extract of *T. indicum* was observed in animal models by Perianayagam *et al*, 2011 b. Reddy *et al*, 2012, have reported the in vitro antioxidant and glucose uptake effects of *T. indicum* in cell lines [31, 32].

Chidambaram *et al*, 2013, have reported the methanolic extracts of *T. indicum* has good antioxidant, antibacterial and antidiabetic activity [33]. Yuldesheva *et al*, 2010 have reported the lipid and lipophilic compounds from seeds of three plants of Boraginaceae family [34].

2. Materials and Methods

2.1. Preparation of extracts

500 grams of dried powder of *Cassia tora* and *Trichodesma indicum* arial parts like, tender shoot, leaves, flowers and fruits were packed in separate round bottom flasks for sample extraction by using different solvents namely chloroform, ethyl acetate, ethanol and hydroethanolic solvents(20% ethanol: 80% distilled water), respectively.

The extraction was conducted with 750 ml of each solvent for a period of 48 hours. At the end of the extraction the respective solvents were concentrated under reduced pressure and the crude extracts powders were stored in refrigerator for further use.

2.2 Phytochemical analysis

The extracts prepared were analyzed for the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, cardiac glycosides and reducing sugars based on the protocols available in the literature [35, 36, 37].

2.2 a. Test for alkaloids

The extract of the crude dry powder of each solvent was evaporated to dryness in boiling water bath. The residues were dissolved in 2 N Hydrocholoric acid.

The mixture was filtered and the filtrate was divided into two equal portions. One portion was treated with a few drops of Mayer's reagent; one portion was treated with equal amount of Dragondroff's reagent, respectively. The creamish precipitate and the orange precipitate indicated the presence of alkaloids.

2.2 b. Test for saponins

About 0.5 g of the plant extract was shaken with water in a test tube and then heated to boil. Frothing was observed which was taken as a preliminary evidence for the presence of the saponin.

2.2 c. Test for tannins

About 0.5 g of extract was added was in 10 ml of water in a test tube and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue-black coloration.

2.2 d. Test for steroids

2 ml of acetic anhydride was added to 0.5 g of methanol extract of each sample with 2 ml sulphuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

2.2 e. Test for flavonoids

2 ml of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution few drops of conc. Hydrochloric acid was added and the red colour was observed for flavonoides and orange colour for flavons.

2.2 f. Test for anthraquinones

About 0.5 g of extract was taken in a dry test tube and 5 ml of chloroform was added and shaken for 5 min. The extract was filtered and the filtrate shaken with equal volume of 10% of ammonia solution. A pink violet or red colour in the ammonical layer indicates the presence of anthraquinones.

2.2 g. Test for cardiac glycosides

0.2 g of extract was dissolved in 1 ml of glacial acetic acid containing 1 drop of ferric chloride solution. This was then under layered with 1ml of concentrated sulphuric acid. A brown ring obtained at the interface indicated the presence of a deoxy sugar characteristic of cardioids.

2.2 h. Test for Proteins

To 2 ml of protein solution 1ml of 40% NaOH solution and 1 to 2 drops of 1% $CuSO_4$ solution was added. A violet color indicated the presence of peptide linkage of the molecule.

2.2 i. Test for Amino Acids

To 2ml of sample was added to 2ml of Ninhydrin reagent and kept in water bath for 20 minutes. Appearance of purple color indicated the presence of amino acids in the sample.

2.2 j. Test for Tri-Terpenoids

5ml of each extract was added to 2ml of chloroform and 3ml of con. H_2SO_4 to form a monolayer of reddish brown coloration of the interface was showed to form positive result for the terpenoids.

2.2 k. Test for Triple Sugar

To 2 ml of extract few drops of Benedict's reagent was added and shaken well. It was heated for 5minutes to observe for any appearance of yellow, orange and red colour to the solution to confirm for the presence of Sugars.

2.3 Antioxidant activity

The antioxidant activities of the two plant extracts were studied using standard DDDH method.

Chemicals & Reagents: 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Methanol, DPPH –1mg/ml in methanol and Ascorbic acid (standard) mg/ml.

Procedure

3.7 ml of absolute methanol was taken in all test tubes along with blank.100µl of absolute methanol was added to the blank. 100 µl of Ascorbic acid was added to the tube marked as standard and 100 µl of respective samples were added to all other tubes marked as tests. Finally, 200 µl of DPPH reagent was added to all the test tubes including blank. All the test tubes were incubated at room temperature and in dark for a period of 30 minutes. The absorbance of all the samples were observed at 517nm.

Calculation for antioxidant activity:

% Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

2.4 Antibacterial activity study

The activities of chloroform, ethyl acetate, ethyl alcohol and hydroalcoholic (20% ethyle alcohol: 80% Distilled Water) extracts of *Cassia tora* and *Trichodesma indicum* were studied against standard antibiotic Penicillin. The results were shown in figure no. 5 (i) and 5 (ii).

2.5 Antifungal activity study

The activities of chloroform, ethyl acetate, ethyl alcohol and hydroalcoholic (20% ethyl alcohol: 80% Distilled Water) extracts of *Cassia tora* and *Trichodesma indicum* were studied against standard antifungal drug, fluconozol. The results

were shown in figure no. 6 (i) and 6(ii).The representative photographs of *Cassia tora* and *Trichodesma indicum* plants are shown in Figure 1, 2.

3. Results

Figure 1. Depicts a twig of *Cassia tora* plant.

Figure 2. Depicts a twig of *Trichodesma indicum* plant.

3 A. Phytochemical Analysis

The study of phytochemicals present in the arial plant parts of *Cassia tora* and *Trichodesma indicum* in various extraction media, namely, chloroform, ethyl acetate, ethanol and hydroethanolic solvents is represented in Table 1 and Table 2, respectively.

Table-1: Phytochemical Analysis of *Cassia tora* arial plant parts.

SL.No.	Phytochemicals	Chlorofom Extract	Ethyl Acetate Extract	Ethanol Extract	(Ethanol+ Water)Extract
1.	Alkaloids	+	-	-	-
2.	Saponins	-	-	-	-
3.	Tannins	+	+	+	+
4.	Steroids	+	+	+	+
5.	Flavonoids	-	-	-	-
6.	Anthraquinones	-	-	-	-
7.	Cardiac glycosides	-	-	-	-
8.	Protein	-	-	-	-
9.	Amino acids	-	-	-	-
10.	Tri-terpenoids	+	+	+	+
11.	Reducing sugar	-	-	-	-

Table .2. Phytochemical Analysis *Trichodesma indicum* arial plant parts.

Sl. No.	Phytochemicals	Chlorofom Extract	Ethyl Acetate Extract	Ethanol Extract	(Ethanol+ Water) Extract
1.	Alkaloids	-	-	-	-
2.	Saponins	-	-	+	+
3.	Tannins	+	+	+	+
4.	Steroids	+	+	+	+
5.	Flavonoids	-	-	-	-
6.	Anthraquinones	-	-	-	-
7.	Cardiac glycosides	-	-	-	-
8.	Protein	-	-	-	-
9.	Amino acids	-	-	-	-
10.	Tri-terpenoids	+	+	+	+
11.	Reducing sugar	-	+	+	+

4. Antioxidant activity results are presented below:

Table 3. Standard Antioxidant table for reference.

S.No	Reagents	Blank	Ascorbic acid				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Incubation at dark for 30 mins							
OD at 517nm		0.63	0.08	0.06	0.05	0.03	0.01
% of antioxidant activity		-	87.3	90.4	92.0	95.2	98.4

Table 3A.The antioxidant activity of chloroform extract of *Cassia tora.*

S.no	Reagents	Blank	*Cassia tora* Chloroform extract (A1)				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Incubation at dark for 30 mins							
OD at 517nm		0.65	0.65	0.64	0.63	0.62	0.59
% of antioxidant activity		-	0.00	1.5	3.0	4.6	9.2

Table 3.B Antioxidant activity of *Cassia tora* Ethyl acetate extract.

S.no	Reagents	Blank	*Cassia tora* Ethyl Acetate extract (A2)				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Incubation at dark for 30 mins							
OD at 517nm		0.65	0.62	0.60	0.58	0.55	0.52
% of antioxidant activity		-	4.6	7.6	10.7	15.3	20.0

Table 3.C Antioxidant activity of *Cassia tora* ethyl alcohol extract.

S.no	Reagents	Blank	*Cassia tora* Ethyl Alcohol extract (A3)				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Incubation at dark for 30 mins							
OD at 517nm		0.65	0.65	0.64	0.62	0.61	0.60
% of antioxidant activity		-	0.00	1.5	4.6	6.1	7.6

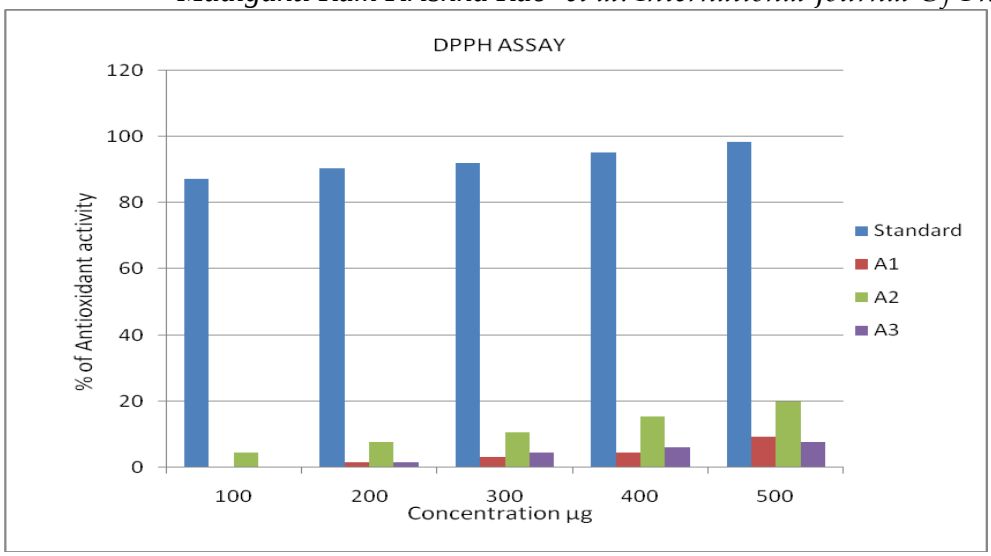

Figure 3 . depicting the antioxidant properties at different concentrations *of Cassia tora* chloroform, ethyl acetate and Ethyl alcohol extracts *(*A1, A2 and A3 respectively*)*

Table 4.A Antioxidant activity of *Trichodesma indicum* chloroform extract.

S.no	Reagents	Blank	*Trichodesma indicum* Chloroform extract (B1)				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Incubation at dark for 30 mins							
OD at 517nm		0.65	0.65	0.65	0.64	0.63	0.60
% of antioxidant activity		-	0.0	0.0	1.5	3.0	7.6

Table 4. B Antioxidant activity of *Trichodesma indicum* ethyl acetate extract.

S.no	Reagents	Blank	*Trichodesma indicum* ethyl acetate Extract (B2)				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl

Incubation at dark for 30 mins						
OD at 517nm	0.65	0.59	0.57	0.56	0.56	0.53
% of antioxidant activity	-	9.2	12.3	13.8	13.8	18.4

Table 4 C. Antioxidant activity of *Trichodesma indicum* ethyl alcohol extract.

S.no	Reagents	Blank	*Trichodesma indicum* Ethyl alchol extract (B3)				
			100 µg	200 µg	300 µg	400 µg	500 µg
1	Methanol Ml	3.8ml	3.7ml	3.7 ml	3.7ml	3.7ml	3.7ml
2	Ascorbic acid ml	-	0.1	0.1	0.1	0.1	0.1
3	Sample µl	-	100 µl	100 µl	100 µl	100 µl	100 µl
4	DPPH ml	200 µl	200 µl	200 µl	200 µl	200 µl	200 µl
Incubation at dark for 30 mins							
OD at 517nm		0.65	0.60	0.58	0.54	0.50	0.47
% of antioxidant activity		-	7.6	10.7	16.9	23.0	27.6

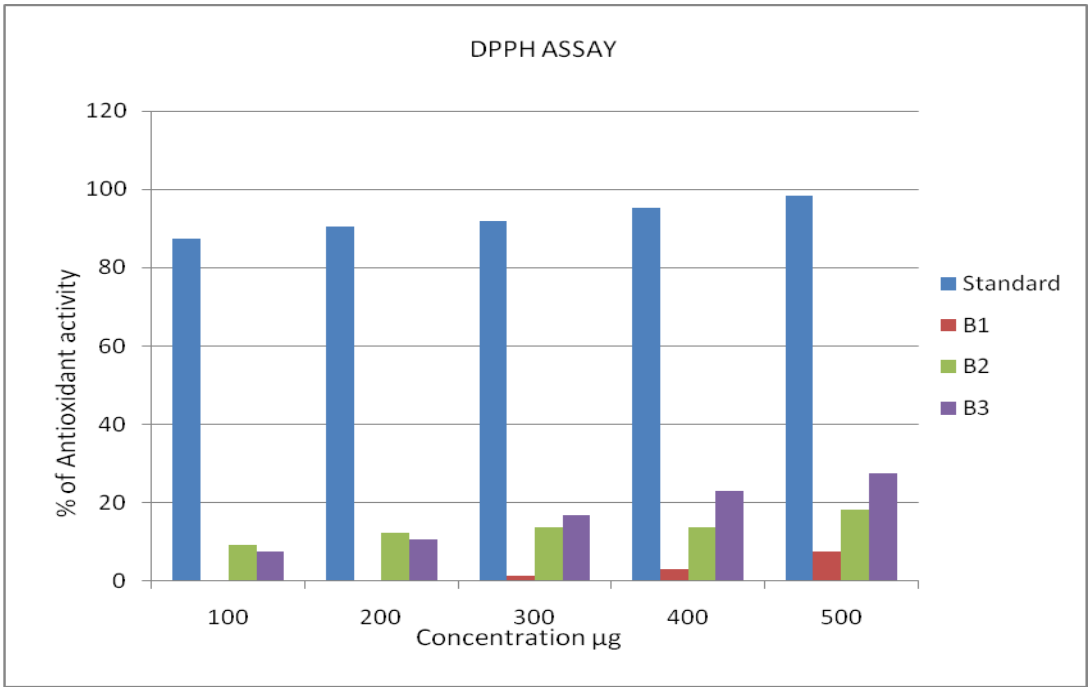

Figure. 4 Depicting the antioxidant properties at different concentrations of *Trishodesma indicum* chloroform, ethyl acetate and Ehtyl alcohol extracts (B1, B2 and B3 respectively)

Table 5. Antibactrial and Antifungal activity Study of *Cassia tora* and *Trichdesma indicum* extracts.

The representative diagrams of the culture plates for antibacterial and antifungal study for both plants are shown in the following Figures (No. C i, C ii and D I and D ii) and Tables No.5

SL No.	ZONE OF INHIBITION						
	Standard Drug (units/disc)	Control	1 disc of drug (units/disc)	Chloroform Extract (In mm)	Ethyl Acetate Extract (In mm)	Ethanol Extract (In mm)	Water: Ethanol (4:1) Extract (In mm)
1.	Penicillin (10 units/disc) (*Cassia tora*)	0	1.5	3.5	5.0	2.0	1.5
2.	Penicillin (10 units/disc) (*Trichodesma indicum*)	0	2.0	1.5	2.0	2.0	1.5
3.	Fluconozole (20 mcg/disc) (*Cassia tora)*	0	1.5	3.5	4.5	3.0	2.0
4.	Fluconozole (20 mcg/disc) (*Trichodesma indicum*)	0	1.2	1.5	2.0	2.0	1.5

The representative diagrams of the culture plates for antibacterial and antifungal study for both plants are shown in the following Figures (No. C i, C ii and D I and D ii).

Fig.C.i. Antibacterial Activity of *Cassia tora* and standard antibiotic penicillin)

Fig.C.ii. Antibacterial Activity of *Trichodesma indicum* and standard antibiotic penicillin)

Fig.D.i Antifungal Activity of *Cassia tora* (standard antifungal agent fluconozole)

FigD. ii. Antifungal Activity of *Trichodesma indicum* (standard antifungal agent fluconozole)

5. Discussion

Our results on the different parameters are discussed below**.** Chavan *et al,* 2011 have reported the presence of glycosides, flavonoids, anthrones, anthracene derivatives, tannins, sugars in ethanolic and water extracts of the *Cassia tora* plant [14]. Kawade and Manisha, 2013 have performed the phytochemical screening of the leaves of this plant [20]. In our study we have found the presence of tannins, steroids and triterpenoids in all the four extracts namely, choloroform, ethyl acetate, ethanol and hydroethanol extracts. It was observed that alkaloids were present only in the chloroform extracts. It was seen that saponins, flavonoids, anthraquinones, cardiac glycosides, proteins, amino acids and reducing sugars were absent in all the extracts of *Cassia tora* arial parts. The antibacterial study of *Cassia tora* was reported by Chavan *et al*, 2011 on *E.coli, B. subtlis, S. aureus* and antifungal studies on *A. niger* and *Candida albicans* with Ciprofloxacin as standard drug [14]. Mukherjee *et al,* 1999, have also worked on the antifungal activity of *Cassia tora* leaf extracts on *C. albicans, A. Niger, S. cerevisae* and *T. mentagophytes* with Griseofluvin as standard drug [21].

In the present study the antibacterial properties were evident by the MIC zones in mm. It was observed that when compared to standard antibiotic, penicillin with a zone of clearance at 1.5 mm, the zones of clearance were 3.5, 5.0, 2.0 and 1.5 mm, in chloroform, ethyl acetate, ethanol and hydroethanolic extracts respectively. These results were very significant and this plant extracts could be used for antibiotic treatment compared to penicillin.

The antifungal activity of *Cassia tora* extracts as shown by the MIC values at 3.5, 4.5, 3.0 and 2.0 mm in chloroform, ethyl acetate, ethanol and hydroethanlic extracts as compared to 1.5 with Fluconozole as antifungal standard. Thus the

antifungal activity of this plant is very significant and further research to develop and promote these plant extracts as antibacterial and antifungal agents.

The antioxidant activity of *Cassia tora* topical cream was reported by Gupta *et al*, 2012 [19]. In our study the maximum antioxidant activity was observed with 500 µg of sample at 20% in ethyl acetate extract while it was 9.2% and 7.6% in chloroform and ethanol extracts, respectively.

The preliminary phytochemical analysis of arial parts of *Trichodesma indicum* has shown the presence of tannins, steroids and triterpenoids in all the extracts. Presence of saponins was observed in ethanol and ethanol water extracts while it was absent in chloroform and ethyl acetate extracts. Similarly, reducing sugars were present in all the extracts except choloform extract. Alkaloids, flavomoids, anthraquinones, cardiac glycosides, proteins and amino acids were conspicuous by their absence. Reddy *et al*, 2012 have reported the antioxidant property of *T. indicum* on cell line cultures [33]. In our study we have found that the antioxidant property was maximum in ethanolic extract at 27.6% followed by ethyl acetate and chloroform extracts at 18.4% and 7.6% respectively with 500 µg of extracts.

Priyanayagam *et al*, 2005, have studied the antibacterial properties of the root extracts of this plant [27]. Saboo *et al* 2013 have demonstrated that ethanolic extract of this plant had antibacterial activity on *B. subtilis* and *S. aureus* whereas the water extract was more effective on *E.coli* [31]. Reddy *et al*, 2012 have reported the antioxidant property of the plant cell lines [33].

In the present study we have observed that the antibacterial activities of different extracts of *T. indicum* did not show much variation as compared to the standard antibiotic, penicillin. The MIC values are 2.0, 1.5, 2.0, 2.0 and 1.5 in standard drug, chloroform, ethyl acetate, ethanolic and hydroethanolic extracts respectively.

The antifungal activities of *T. indicum* also did not show any significant variation when compared with standard drug, fluconozole. The MIC zone of clearance was 1.2, 15, 2.0, 2.0 and 1.5 mm in standard drug, chloroform, ethyl acetate, ethanolic and hydroethanolic extracts respectively.

From the above results and discussion it was evident that among the two medicinal plants, namely, *Cassia tora* and *Trichodesma indicum*. *Cassia tora* indicated prominent anti bacterial and antifungal properties in almost all the extracts where as *Trichodesma indicum* did not reflect the same. It was also observed that the antioxidant properties of different extracts of *Cassia tora* was not that promising as compared to *Trichodesma indicum.*

6. References

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