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Antimicrobial potential and phytochemical screening of leaf extracts in Cassia tora l.

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Abstract : Recently, Antimicrobial potential and phytochemical screening of leaf extracts of Cassia toral. from the campus of Government Vidharbha Institute of Science and Humanity, Amravati had been investigated. Leaf sample contained tannin, saponin, protein, steriods, carbohydrate, alkaloids, flavonoids and glycosides. Also existence of some phytochemicals like tannin, saponin and steroids illustrated medicinal action of the plant in its therapeutic uses. Present investigation showed that leaf extract of Cassia tora l. are good source of bioactive compounds which have some medicinal applications. It was studied against four different bacteria by disc diffusion method. Antimicrobial activity was evaluated again human pathogens E. coli, P. vulgaris, S. typhi and S. aureus using an agar diffusion array. The leaves crude extract showed minimum inhibitory concentration of 385mg/ml. for S. typhi. The result of their phytochemcial screening and antimicrobial activity could justify the observed activities and validate their use in herbal medicine and can also be employed in the treatment of various ailments in modern medicine too.

Keywords: Phytochemical, Antimicrobial analysis, Cassia tora l.

I. INTRODUCTION

Nature has been a source of medicinal agents since time immemorial. Plants play a significant role in providing primary health care. They serve as therapeutic agents as well as important raw materials for the manufacturing of traditional and modern medicines as well as in mainly due to the current widespread belief that green medicine are a safer and more dependable than the costly synthetic drugs mainly of which have adverse side effect [1].

Traditional medicine holds a great source of easily available effective therapy for skin diseases to the people, particularly in tropical developing countries, including India.

People used several plant derived preparations to cure skin diseases. Cassia tora l. is the very common Indian herbs having various medicinal properties for the treatment of different kinds of disease [2].

For the treatment of various diseases, globally herbal medicines have been used traditionally. In the last decade, study of plant extract has attracted attention in curing various challenging diseases. In the indigenous system of medicine Cassia tora l. is one of the plants that areuse for many centuries. Different parts of the plant root, seeds, leaves and stem have medicinal values.

Exhaustive literature survey reveals that only proximate and phytochemcial analysis of stem and seeds of Cassia tora l. have been investigated but phytochemical analysis of leaves extract by various solvent is still lacking hence this work had been carried out.[3]

Cassia toral. is an annual foetid herb with a height of 28cm to 100cm. It is found in Asian region but in India it is mainly found in Satpura region in Maharashtra. Cassia tora l.is very stress tolerant and is an easy plant to grow in India; it occurs as wasteland rainy season weed. Seeds extract can be used as energy drink.

Phytochemical are chemical compounds derived from plants that are non-nutritive secondary metabolic compounds occurring in different parts of plants. They are important as protective and disease fighting compounds which help the body to prevent of fight against diseases and so are required by the human body to sustain life. Their therapeutic use in prevention or fighting a number of diseases is the basis of their extensive use in traditional medicine. Some of the phytochemicals are water soluble while others are not[4,5].

II. MATERIALS AND METHODS

A. Plant Collection And Preparation

Leaves of Cassia tora l. were collected from campus of G.V.I.S.H. Amravati, Maharashtra. They were properly shade dried indoors in an airy place, crushed, powdered and stored in dry opaque bottles.Leaf was extracted with ethanol-benzene in a soxhlet apparatus. Solvent was evaporated and the resultant extract was stored at 20 °C until use.

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B. Method of Extraction for CASSIA tora l.

Extraction of the leaf of Cassia tora l. was carried out by using ethanol-benzene soxhlet extraction technique. About 5gm of coarsely powdered leaf was sequentially extracted in a soxhlet extractor using 400ml ethanol-benzene. Extraction time was four hours for each solvent. Resulting extracts were evaporated using rotary evaporator. Filtrates were then combined, concentrated to dryness under controlled temperature and pressure [6, 7].

C. Phytochemical Analysis

Phytochemicals in the leaves was determined by elemental analysis of magnesium, calcium, sulphur, iron, sodium and chlorine were investigated by color test using appropriate chemicals and reagents and also filtrate used to test for phenols, tannins, saponins, glycosides, flavonoids, steroids and alkaloids[8,9,10].

Phytochemical analysis was carried out for the leaf extract as per standard methods [11, 12].

1) *Detection of alkaloids by Mayer's test: Filtrate* was treated with Mayer's reagent. Formation of yellow cream precipitate indicates the presence of alkaloids.
2) *Detection of tannin by Gelatin test: To* the extract 1% gelatin solution containing sodium chloride was added, formation of white precipitate indicates presence of tannin.
3) *Detection of saponin by Frothtest:* Extract was diluted with dist. Water to 30ml and this was shaken in a graduated cylinder for 20minutes, formation of layer of foam indicates the presence of saponin.
4) *Detection of flavonoids by Lead acetate test: N* Extract was treated with few drops of lead acetate solution; formation of yellow color precipitate indicates presence of flavonoids.
5) *Detection of Carbohydrate by Molisch'stest:* Filtrate was treated with 2 drops of alcoholic α-naphtholsolution in a test tube and 2ml of conc. sulphuric acid was added carefully along the sides of the test tube, formation of violet ring at the junction indicates presence of carbohydrate.
6) *Detection of protein by Xanthoproteictest: Extract* was treated with few drops of concentrated Nitric acid solution; formation of yellow color indicates presence of protein.
7) *Detection of steroids by Libermann Burchard'stest:* Extract was treated with chloroform and filtered then filtrate was treated with few drops of acetic anhydride, boiled it and then cooled. Conc. sulphuric acid was added carefully to the test tube, formation of brown ring at the junction indicates the presence of steroids.
8) *Detection of glycosides by Legal'stest:* Extract was treated with sodium nitropursside in pyridine; formation of pink to blood red color indicates presence of glycosides.

D. Antimicrobial Activity

Antimicrobial activity was carried out using the agar disc diffusion method. Negative controls were prepared by using same solvents employed to dissolve the samples. Inhibition zones were measured and compared with the standard reference antibiotic amoxicillin. Each extract was subjected to serial dilution by using dimethyl sulphoxide (DMSO) as a solvent to give 2 mg/ml solution. Concentration of amoxicillin standard used for this study was at 2 mg/ml. prepared concentration of extract was tested for its antimicrobial activity against 1 gm. positive bacteria and 3 gm. negative bacteria on nutrient agar plates using disc diffusion method. All the plates were incubated at 37 °C for 24 hr. Evaluation of antimicrobial activity was measured showing the diameter of the zones of inhibition against the tested bacteria.



Cassia tora l. plant

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Cassia tora l. leaves



Cassia tora l. leaves powder

III. RESULTS

A. *Phytochemical analysis of the Cassia toral. leaf.*

Sr. No.	Content	Test	Result
1	Alkaloids	Mayers test	+
2	Tannin	Gelatin test	+
3	Saponin	Froth test	+
4	Flavonoids	Lead acetate test	+
5	carbohydrate	Molish test	+
6	Protein	Xanthoproteic test	+
7	Steroids	Libermann -Burchard test	+
8	Glycosides	Legal's reagent	+

"+" = present, "-" = absent

B. *Nutrient Analysis of Cassia tora l. leaf*

Sr. No.	Content	Result
1	Magnesium	+
2	Calcium	+
3	Sulphur	+
4	Iron	+
5	Sodium	+
6	Chlorine	+
7	Potassium	+

"+" = present, "-" = absent

Total four isolated compounds were studied for their antimicrobial activities. All the pathogens tested during analysis are human pathogen. Activities of compounds were tested against all the pathogens by disc diffusion method. It was found that all the compounds are active against bacteria.

C. *Antimicrobial Activity of Compounds*

Comp.	*E. coli*	*P. vulgaris*	*S. typhi*	*S. aureus*
P1	Active	Inactive	Active	Active
P2	Active	Inactive	Active	Active
P3	Active	Inactive	Active	Active
P4	Active	Inactive	Active	Active

D. *Minimum Inhibitory Concentration (MIC) values of active compounds in mgml^{-1}*

Comp.	*E. coli*	*P. vulgaris*	*S. typhi*	*S. aureus*
P1	810	3890	812	1090

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P2	745	3722	798	1823
P3	440	3596	385	768
P4	1533	3539	2232	2337

MIC value = 3500-1900 Inactive;
1800-1500 Weakly active;
1400-1000 Moderately active;
< 1000 Highly active.

IV. DISCUSSION

Cassia toral. leaf contains lower fiber content [11]. Proteins are also found in good proportion and they are important and act as enzyme, hormones and antibodies, proteins also helps in the formation of bones, hair and it contributes less energy than 30calories and thus prevents obesity and other related disease. A diet of fat providing 1-2% is sufficient for a human being. High amount of carbohydrates is essential for maintenance of life in plant and animals and also provide raw material for many industries [14].
Presence of flavonoids inferred that the leaf has the biological functions like antioxidant, allergies protection, free radical, inflammation, ulcers, hepatotoxins, tumor and viruses [15]. Flavonoids are water soluble free radical and antioxidants which prevent oxidative cell damage, and have strong anti-ulcer and anticancer activity [16]. Saponin content suggests that usefulness of the leaf as a productivity agent. Saponin level is low, either compared with the results from another works. Alkaloids are the most efficient medicinally significant bioactive substances in plants. Alkaloids and the synthetic derivatives are used as medicinal agents because of their bactericidal and analgesic properties. These are water soluble phenolic compounds which precipitate proteins. They exist in all plants. Tannins add to proteins making them bio-unavailable [17, 18].
Potassium was the most abundant element. Potassium helps to control body weight and improve water and electrolyte balance in the blood and tissues. Calcium content was determined. It helps in the improvement of muscle contraction required by infants and fetuses for bones and teeth development [19].Concentration of sodium was low; this vegetable is useful in the treatment of heart related diseases. Excess sodium utilization leads to hypertension. Iron is an important element in the diet of pregnant women, nursing mothers, infants and the elderly to prevent anemia diseases. Magnesium also plays necessary roles in most reaction involving phosphate transfer [20]. It is important in the structural stability of nucleic acids. It plays a powerful role in the internal absorption of electrolyte in the body. Its defect in man includes severe diarrhea and migraines [21, 22].

A. Activity against E. Coli

E. coli is a gram negative parasite living only in human or animal intestine.Clinical infections caused by *E. coli* were urinary tract infection, diarrhea, pathogenic infection and septicemia. Generally the patients of diarrhea were observed in February to July.
It can be easily seen that the compounds P1, P2 and P3 showed highly activity in minimum concentration while, P4 compound are moderately active. So these synthesized drugs can be used as the best alternative drugs for the treatment of diseases caused by *E. coli*, only after the pharmaceutical, biochemical and medicinal significance, if these drugs do not have adverse and other toxic effects [23, 24, 25, 26].

B. Activity against S. aureus

S. aureus is a gram positive bacterium found in wounds and is a causative agent of wound infection. It occurs in clusters like grapes. Its ability to develop resistance to penicillin and other antibiotics enhances its importance as human pathogen. It produces two types of diseases infection and intoxication. Since such type of bacteria develop resistance to common antibiotics, newer and newer types of drugs will have to be always synthesized and tested against them.
Four compounds are tested against S. aureuspathogen from which P3 are highly active, P1 are moderately active while P2 and P4 is weakly active. These active compounds can be used for treatment of wound infections, after biological, pharmaceutical, medical study and if these do not have any toxic effects[23,24,25,26].

C. Activity AgainstS. typhi

S. typhiis a gram negative bacterium causative agent of typhoid ranging from 7-14 days. Patient shows mild pyrexia which may become fatal fulminating disease. As bile is good culture medium for the bacteria, it is multiplied abundantly in gall bladder, and is discharged continuously into intestine where it involves the Teyer's patches and lymphoid follicles of the illium.

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Four compounds are tested against *S. typhi*pathogen from which P1, P2 and P3 are highly active while P4 is weakly active. So these synthesized drugs can be used as an alternative drug for the treatment of disease caused by *S. typhi*only after their detailed study in pharmaceutical, biochemical and medicinal sciences. These drugs may replace the traditional drugs if they do not have toxic and other side effects [23,24,25,26].

D. Activity against P. vulgaris

Proteus vulgarisis a gram negative bacterium that inhibits the intestinal tracts of humans and animals. It can be found in soil, water and fecal matter. It is an opportunistic pathogen of humans and it is known to caused urinary tract infections and wound infections. Four compounds are tested against *P. vulgaris* pathogen which all is inactive [23,24,25,26].

V. CONCLUSION

This type of study will be applicable for the pharmaceutical, medicinal, agricultural, industrial and biochemical sciences. This study also showed that proximate, phytochemical, mineral analysis of *Cassia toral* .leaf as a balanced and rich source of macro- and micronutrients. The plant was also used by rural people as a vegetable in winter that's mean it was used as heat and energy. So the further study will be carrying out on this plant.

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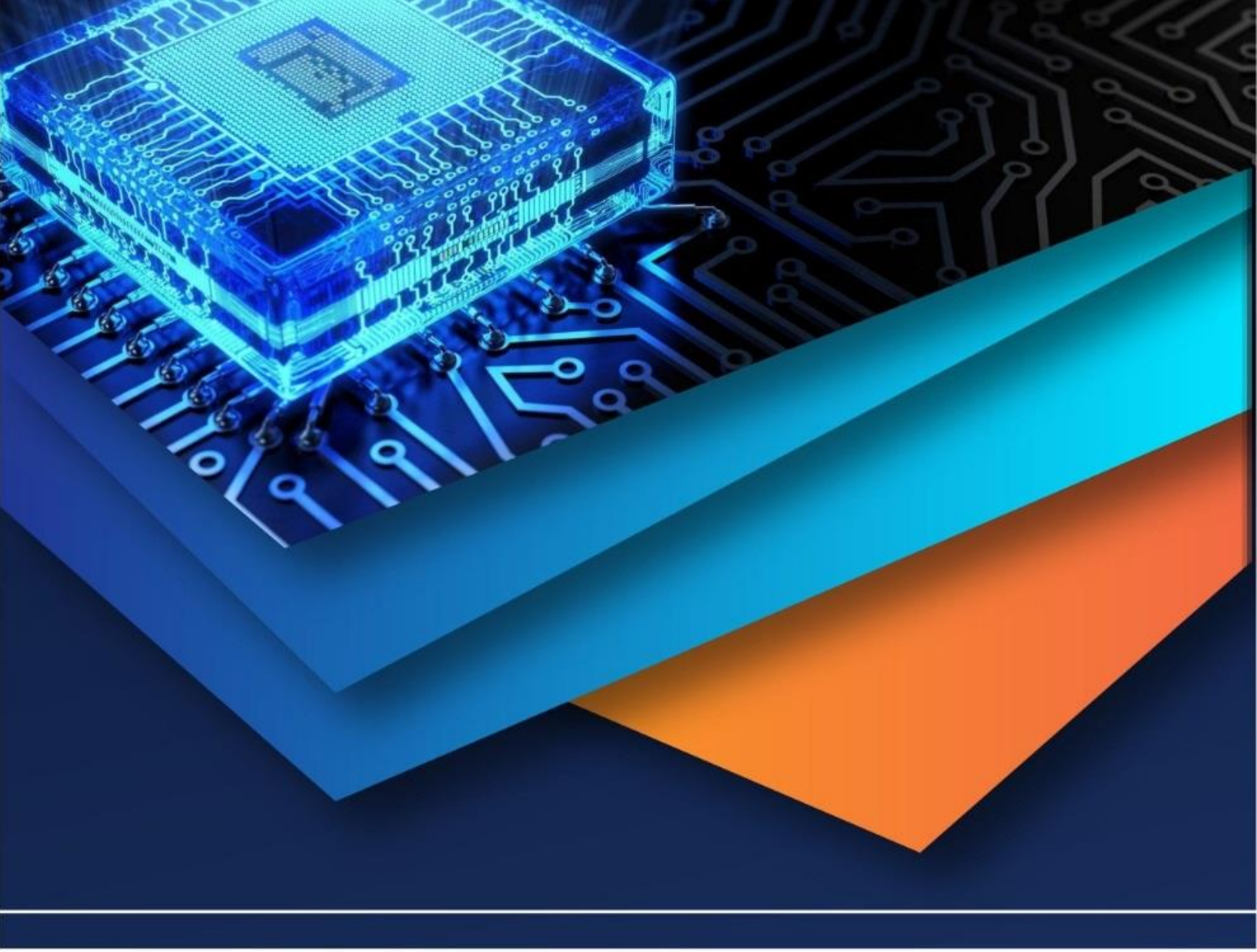

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