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Evaluation of Antimicrobial Activity & Phytochemical Analysis of *Cassia siamea*

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Abstract

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. Millions of rural households use medicinal plants in a self-help mode. *Cassia siamea* is an ornamental tree of the family Caesalpiniaceae having ethno-medicinal importance. Ethanolic, methanolic and aqueous extracts of leaves of *Cassia siamea* were investigated for its antimicrobial activity against *Escherichia coli (2065), Bacillus subtilis (2063), Staphylococcus aureus (2602), Pseudomonas aeruginosa (2036), Micrococcus luteus-(2103), Aspergillus niger* (NCIM-920), *Fusarium moniliform* (NCIM-1099), *Candida albicans* (NCIM-3102), *Aspergillus flavus* (NCIM-540) and *Alternaria alternata* (NCIM-718) The ethanolic and methanolic extract showed effective inhibitory activity as compared to aqueous extract. Results of phytochemical investigations of leaves of *C. siamea* indicates the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, amino acids, terpenoids and steroids in all the three solvents. While glycosides were present in methanol and ethanol, and anthraquinones were present in ethanol and water.

Keywords: *Cassia Siamea*, Traditional, Antimicrobial Activity, Phytochemical Investigations.

Introduction

Plant remain an important resource to cure serious diseases in the world. The traditional medicinal methods, and more significantly, the use of medicinal plants, play an important role to fulfill the basic health needs in the developing countries. The medicinal value of these plant materials lies in some chemically active substances that produce a definite physiological action on the human body. The most active of these bioactive constituents of these ethnomedicinal plants are alkaloids, tannin, flavonoid and phenolic compounds. [Edeoga et. al., 2005]

Plants are considered not only as dietary supplement to living organisms but also traditionally used for treating many health problems and the medicinal value of many plants still remains unexplored investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents. [Anushia,et. al.,] Plant-derived substances have recently become of great interest owing to their versatile applications. Harborne (1998) was the pioneer who proposed the phytochemical methods.

Materials and Methods

Antimicrobial activity

The fresh plant materials have been collected from the local areas of Sagar district of M. P. The plant specimen was botanically identified and authenticated by comparing the herbarium specimen available in the Department of Botany, Dr. H. S. Gour University, Sagar. The plant parts were washed in tap water, shade dried, powdered in a kitchen blender and was stored in air tight plastic bags.

Extraction

It was performed by hot extraction method using Soxhlet apparatus. For extraction powdered plant material (~25g) was packed in Whatmann filter paper No. 1 and introduced into the Soxhlet apparatus. Successive extraction was performed with different solvents on basis of their polarity (ethanol, methanol, water). After each extraction the drug packet was air dried and then re-introduced for extraction with the successive solvent, while the respective extracts were concentrated and dried in a water bath maintained at 65-70`C. Dried extracts were transferred to air tight bottles and stored at 4`C.

For bacteria, autoclaved nutrient agar media was poured into sterile petri plates. The plates were allowed to set for an hour. A standardized bacterial inoculums was introduced in to the surface of sterile agar plates. Sterile paper discs previously soaked in the extracts were introduced into the labeled plates. The plates were incubated at 37C for 24 hours.

For fungi, autoclaved Potato dextrose agar media was poured into sterile petri plates. The plates were supplemented with plant extracts. A standardized fungal inoculums was introduced in to the centre of sterile PDA plates. The plates were incubated at 27C for 2-4 days. The absence or slow growth was regarded as the presence of antifungal activity. An inoculated plate without the plant extract was taken as control.

Test Organisms

Bacteria

The bacterial culture were obtained from National Collection of Industrial Micro-organism (NCIM) Pune. Their accession no. with scientific name and specific properties are given below:

S. No.	Scientific Name	Accession No. of NCIM
1	*Escherichia coli* (Migula)	2103
2	*Bacillus subtilis* (Ehrenberg)	2501
3	*Pseudomonas aeruginosa* (Schroter)	2063
4	*Sarcina lutea* (Schroeter)	2493
5	*Staphylococcus aureus* (Rosenbach)	2065

Fungi

The fungal strains were obtained from National Collection of Industrial Micro-organism (NCIM) Pune. Their accession no. with scientific name and specific properties are given below:

S. No.	Scientific Name	Accession No. of NCIM
1	*Aspergillus niger* (Van Tiegh.)	920
2	*Aspergillus flavus* (Fr. Link.)	540
3	*Alternaria alternata* (Fr. Keissl.)	718
4	*Fusarium sp.* (J. Sheld.)	1099
5	*Candida albicans* (C. P. Robin)	3102

Preparation of media-There are so many methods for studying anti-bacterial and anti-fungal activities. In the present research work filter paper disc diffusion method (Rober, 1970, Verma & Imann, 1973) would be followed and the media used are given below-

Nutrient culture media used for anti-bacterial activity

Oxide nutrient agar medium having following composition was used for preparing the slants and plates-

Beef extract - 3g

Peptone - 10g

Glucose - 25g

Agar agar powder - 20g

Distilled water to make the solution 1liter.

Nutrient culture media used for anti-fungal activity-

Potato dextrose agar medium would be used for making the inoculums and the medium was prepared by taking the following composition of substances:

Potato slices - 200g

Dextrose - 25g

Agar agar powder - 20g

Distilled water to make the solution 1litre.

All the components were dissolved in freshly prepared distilled water. The flask were plugged very well with cotton and sterilized in an autoclave at 151bs pressure for 30 minutes.

Procedure for performing the Disc Diffusion test (Bayer *et al*., 1966): The required amount of Petri plates were prepared and autoclaved at 121°C for 15 minutes. They were allowed to cool under Laminar air flow. Aseptically transfer about 20 ml of media into each sterile Petri dishes and allowed to solidify. 1 ml inoculum suspension was spread uniformly over the agar medium using sterile glass rod to get uniform distribution of bacteria. The readily prepared sterile discs were loaded with different plant extract. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5°C for 1 hour to permit good diffusion and then transferred to an incubator at 37°C and 28°C for 72hours and 24 hours for antifungal and antibacterial activity respectively. The antimicrobial activity was recorded by measuring the width of the clear inhibition zone around the disc using zone reader (mm).

Zone of inhibition of antibacterial activity of Streptomycin the standard drug for different bacteria tested are:

Escherichia coli - 28 mm

Bacillus subtilis - 24mm

Pseudomonas aeruginosa - 30 mm

Sarcina lutea - 32 mm

Staphylococcus aureus - 20mm

The values of diameter include the diameter of 6mm of filter paper disc.

Zone of inhibition of antifungal activity of Grassioflavin for different fungi tested are-

Aspergillus niger - 21mm

Aspergillus flavus - 20 mm

Alternaria alternata - 24 mm

Fusarium monilifer - 22. 5 mm

Candida albicans - 20mm

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Phytochemical Studies

Materials & Methods

Collection of Plant material

The fresh plant materials have been collected from the local areas of Sagar district of M. P. The plant specimen was botanically identified and authenticated by comparing the herbarium specimen available in the Department of Botany, Dr. H. S. Gour University, Sagar. The plant parts were washed in tap water, shade dried, powdered in a kitchen blender and was stored in air tight plastic bags.

Phytochemical analysis: The dried plant parts (leaves) were ground into uniform powder and stored in containers. The dried plant parts were subjected to qualitative chemical screening for the identification of various classes of active chemical constituents using standards prescribed methods (Harborne, 1973, Trease and Evans, 1978 Sofawara 1993, Uddin, et, al,. 2011 and Savithramma, et, al,. 2011).

Extraction

It was performed by hot extraction method using Soxhlet apparatus (Aguwa et al., 1997). For extraction powdered plant material (~25g) was packed in Whatmann filter paper No. 1 and introduced into the Soxhlet apparatus. Successive extraction was performed with different solvents on basis of their polarity (methanol, ethanol, water). After each extraction the drug packet was air dried and then reintroduced for extraction with the successive solvent, while the respective extracts were concentrated and dried in a water bath maintained at 65-70˚C. Dried extracts were transferred to air tight bottles and stored at 4˚C. All the extracts of the selected plant parts were screened for their phytoconstituents in order to see the chemical nature of the compounds present in the extracts. A portion of residue from each extract was subjected to phytochemical analysis in order to see the presence of sterols, alkaloids, carbohydrates, tannins, phenols etc in the extracts. (Sofowora A., 1993)

Later the percentage yield was calculated using following formula-

$$\% \text{ Yield} = \frac{\text{Weight of extract}}{\text{Dried weight of powder material}} \times 100$$

Phytochemical screening: Phytochemical examinations were carried out for all the extracts as per the standard methods. The screening was done only for the presence or absence of the phyto-chemicals. No detailed studies were performed.

1. **Detection of alkaloids:** Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) **Mayer's Test:** Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

b) **Wagner's Test:** Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) **Dragendroff's Test:** Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

d) **Hager's Test:** Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. **Detection of carbohydrates:** Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

b) **Benedict's Test:** Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

c) **Fehling's Test:** Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. **Detection of glycosides:** Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

a) **Modified Borntrager's Test:** Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink colour in the ammonical layer indicates the presence of anthranol glycosides.

4. **Legal's Test:** Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

5. **Detection of saponins**

a) **Froth Test:** Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) **Foam Test:** 0. 5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

6. Detection of phytosterols

a) **Salkowski's Test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

b) **Libermann Burchard's test:** Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids

a) **Alkaline Reagent Test:** Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) **Lead acetate Test:** Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

10. Detection of proteins and amino-acids

a) **Xanthoproteic Test:** The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

b) **Ninhydrin Test:** To the extract, 0. 25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

11. Detection of Diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes (Roopashree, et. al., 2008)

12. Detection of Anthraquinones

1ml of the filtrate with 10ml benzene, filter and now add 5ml of 10% (v/v) ammonia to the filtrate and shake well. Development of pinkish colored solution indicated the presence of anthraquinones.

13. Terpenoids

Take 1ml of the filtrate with 2ml CHCl3 and carefully add few drops of conc. H2SO4. An interface with a reddish brown coloration is formed.

Result & Discussion

Table 1. 1-1. 3 showed the antibacterial activity of *Cassia siamea* plant parts viz. . root, stem, leaf and seeds in water, ethanol and methanol extracts against five bacterial strains were compared to Streptomycin (standard). All the extracts have exhibited different degrees of antibacterial activity against the tested bacteria; among them leaf extracts showed broad spectrum activity against all the test pathogenic bacteria. The ethanol leaf extract of *C. siamea* showed highest inhibition zone against *B. sublitis* (19. 25mm) followed by *S. aureus* (18mm), *S. lutea* (16mm) and *P. aeruginosa* (15. 5mm) while *E. coli* showed least inhibitory activity. The methanolic leaf extract showed strong inhibitory activity against *S. aureus* (20mm) and *S. lutea* (20mm) followed by *B. sublitis* (16mm), *P. aeruginosa* (13mm)and *E. coli* (12mm). In aqueous leaf extract the growth of inhibition was best in *B. sublitis* (18mm)followed by *S. lutea* (15mm), *S. aureus*(14. 5mm) and *P. aeruginosa* (12. 25mm) while *E. coli* showed least inhibitory effect. A similar work was done by Bhadouria, et. al., 2011 reported that the extract of leaves of *Cassia siamea* was the most effective inhibitor against *S. aureus*. All the other samples of root, stem and seed had either shown very negligible inhibitory activity or showed no growth of inhibition against all the bacterial strains tested.

Zone of inhibition of antifungal activity of Grassioflavin for different fungi tested are-

As per gillus niger - 21mm

As per gillus flavus - 20 mm

Alternaria alternata - 24 mm

Fusarium monilifer - 22. 5 mm

Candida albicans - 20mm

Table 1. 4-1. 6 presented the antifungal activity of *Cassia siamea* plant parts viz. . root, stem, leaf, seed in water,ethanol and methanol extracts against five fungal strains. Ethanolic extract of leaf of *C. siamea* showed highest activity against *A. niger* (19. 5mm) and *A. flavus* (17. 5m) followed by *C. albicans* (16. 75mm), *F. monilifer* (14. 25mm) while least activity was

seen in *A. alternata.* In methanolic leaf extract the maximum inhibition zone was seen in *A. niger* (18mm) and *C. albicans* (17. 5mm) followed by *A. flavus* (16. 5mm) and *F. monilifer* (15mm) whereas minimum was seen in *A. alternata*. The study made on aqueous leaf extract showed highest inhibitory activity against *A. flavus* (15mm) and *C. albicans* (13mm) followed by *F. monilifer* (12. 5mm) and *A. alternata* (11. 5mm) while least was shown in *A. niger*. A similar work was done by Chanda, et. al., 2012, reported that leaves of *C. siamea* possess good antifungal activity. All the other samples of stem and seed had either shown very negligible activity or showed no activity against all the five fungal strains.

In case of *Cassia siamea* leaves the highest percentage yield was obtained in aqueous 3. 1% and the lowest in ethanol 2. 1%(6. 10). Results of phytochemical investigations of leaves of *C. siamea* indicates the presence of alkaloids, carbohydrates, saponins, tannins, flavonoids, amino acids, terpenoids and steroids in all the three solvents. While glycosides were present in methanol and ethanol, and anthraquinones were present in ethanol and water(6. 23). Wiam,et. al., (2005)found that ethanol extract of *Cassia siamea* leaves indicated that it contained alkaloids, glycosides, anthraquinones, steroids, tannins and saponins. Mohammed,et. al., (2013) were done the preliminary phytochemical screening indicated the presence of flavonoids, tannins, polyphenols, anthraquinones, saponins, and glycosides.

Table 1.1.Antibacterial activity of aqueous extract of *Cassia siamea*

Plant part used	***E. coli***	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***S. lutea***
Root	_	_	_	_	_
Stem	6mm	_	_	_	_
Leaf	10mm	18mm	14. 5mm	12. 25mm	15mm
Seed	_	7mm	_	6. 5mm	_

Table 1.2.Antibacterial activity of ethanolic extract of *Cassia siamea*

Plant part used	***E. coli***	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***S. lutea***
Root	_	_	_	_	_
Stem	_	_	_	_	_
Leaf	10. 75mm	19. 25mm	18mm	15. 5mm	16mm
Seed	6. 5mm	_	8mm	_	_

Table 1.3.Antibacterial activity of methanolic extract of *Cassia siamea*

Plant part used	***E. coli***	***B. subtilis***	***S. aureus***	***P. aeruginosa***	***S. lutea***
Root	_	_	_	_	_
Stem	8mm	7. 75mm	_	_	_
Leaf	12mm	16mm	20mm	13mm	20mm
seed	8. 5mm	_	8. 5mm	_	_
Streptomycin (standard)	28mm	24mm	20mm	30mm	32mm

_: shows no activity.

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The values of diameter include the diameter of 6mm of filter paper disc.

Table 1.4.Antifungal activity of aqueous extract of *Cassia siamea*

Plant part used	*A. niger*	*Fusarium*	*C. albicans*	*A. flavus*	*A. alternata*
Root	_	_	_	_	_
Stem	_	_	_	8. 75mm	6mm
Leaf	10mm	12. 5mm	13mm	15mm	11. 5mm
seed	6mm	7. 5mm	_	_	_

Table 1.5.Antifungal activity of ethanolic extract of *Cassia siamea*

Plant part used	*A. niger*	*Fusarium*	*C. albicans*	*A. flavus*	*A. alternata*
Root	_	_	_	_	_
Stem	_	_	_	9mm	7mm
Leaf	19. 5mm	14. 25m	16. 75mm	17. 5mm	13. 25mm
Seed	7. 5mm	9mm	_	_	_

Table 1.6.Antifungal activity of methanolic extract of *Cassia siamea*

Plant part used	*A. niger*	*Fusarium*	*C. albicans*	*A. flavus*	*A. alternata*
Root	_	_	7mm	_	_
Stem	_	_	_	8mm	8. 5mm
Leaf	18mm	15mm	17. 5mm	16. 5mm	13. 75mm
Seed	6. 5mm	10mm	_	_	_
Grassioflavin (standard)	21mm	22. 5mm	20mm	20mm	24mm

_: shows no activity.

The values of diameter include the diameter of 6mm of filter paper disc.

Table 1.7.Percentage yield of different extracts of *Cassia siamea* leaves

S. No.	Solvent	Color and consistency	Percentage yield of extraction (% w/w)	Dry weight of the extraction
1	Ethanol	Dark Green(solid)	2. 1	1. 5 gm
2	Methanol	Light Brown (solid)	2. 9	2. 3 gm
3	Aqueous	Green (semi solid)	3. 1	2. 7 gm

Table 1.8.Phytochemical analysis of *C. siamea* leaves

Tests / Extracts	Methanol	Ethanol	Water
Alkaloids Dragendorff's & Mayer's reagent	+	+	+
Carbohydrates Molish's test & Fehling's test	+	+	+
Glycosides Fehling's test	+	+	-
Saponins Froth test	+	+	+
Phenols	-	+	+
Tannin	+	+	+
Flavonoids Shinoda test	+	+	+
Amino acids Ninhydrin test	+	+	+
Terpenoids	+	+	+
Anthraquinone Borntrager's test	-	+	+

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