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Research Article

Assessment of Phytochemical Evaluation and *In-vitro* Antimicrobial Activity of *Cassia angustifolia*

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ABSTRACT

Cassia angustifolia, belongs to *Fabaceae* family. The genus cassia has traditional medicinal value to cure various illness caused by various pathogens. The present work is aimed to reveal the phytochemical evaluation and *in vitro* antimicrobial activity of non polar to polar extracts of *Cassia angustifolia*. In these work the results proved that *Cassia angustifolia* extracts possess a significant amount of secondary metabolites. Methanol is most effective solvent to extract the metabolites from the *Cassia angustifolia.* This also said that all the extracts possess detectable amount of phenols and flavonoids. The methanol extract possess high amount of phenols and flavonoids followed by water, acetone and hexane extract. The extracts also show antimicrobial activity against various pathogenic bacteria and fungi. Out of which methanol extract of *Cassia angustifolia* shows most effective antimicrobial activity followed by water, acetone and hexane extracts. Further work is needed to improve plant based drugs from *Cassia angustifolia.*

Key words: *Cassia angustifolia*, Secondary metabolites, phenols, flavonoids, antioxidant activity.

INTRODUCTION

In Indian culture, medicinal plants are valuable resources used to cure varieties of human and animal diseases, especially in the rural areas where modern/allopathic medicine is impracticable and unaffordable. India has one of the most diverse geographical floras in the world and has a cultural diversity with traditional healing being integral to each ethic group.

In modern era antibiotics are the primary drugs used in the treatment or elimination of several pathogens from the infested/ effected host[1]. The development of antibiotic resistant pathogens against the conventional drugs result in fresh challenges because infectious diseases previously under control are re-emerging with more virulence[2]. Therefore, there is an urgent need for discovery and development of new drugs with high efficacy against the pathogens and minimal side effects to the host. Plants and plant based preparations have been used in traditional practice to treat various kinds of diseases. Plant derived compounds could use a different mechanism of fighting pathogens from established antimicrobials and also possess a clinical value in treating diseases caused by resistant strains of pathogens[3]. The present work is carried out to assess the qualitative and quantitative analysis of phytochemical and in vitro antimicrobial activity of *Cassia angustifolia* polar and non polar solvent extracts.

Cassia is the largest genus of subfamily Caesalpinioideae, belongs to Leguminasae family. It comprises about 600 species[4-5]. Members of the genus Cassia consists of annual or perennial herbs, shrubs and trees which have been differentiated on the basis of number of leaflets, fertile and sterile stamens in single flower and glands present on the leaves. Many plants of Genus Cassia are widely used in traditional medicine and highly valued in industries as they possess various pharmacological activities[6].

MATERIALS AND METHODS:

Collection of plant material

The plant material was collected from the Seshachalam forest and it was authenticated by taxonomic expert Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University (SVU), Tirupathi, Andhra Pradesh. Required quantity of plant raw material i.e. leaves of *Cassia angustifolia*, were collected and washed with running water followed by distilled water. Chopping process was carried out by separating the leaves from stems and they wereallowedto dry under shade[7]. The dried material was stored in asterilized polythenebagsfor further study.

Extraction technique

The dried powder of the leaves was extracted sequentially[8] by soxhletapparatus[9], using different solvents depending upon their polarities like Hexane, Acetone, Methanol and water. The extracts were concentrated, using rotary evaporator. The dried crude concentrated extracts were weighed to calculate the extractive yield and stored in a air tight bottles, until used for analysis.

Phytochemical Analysis

Preliminary screening of Phytochemicals (Qualitative analysis)

Standard screening tests of four extracts *of Cassia angustifolia*, were carried out to know the presence /

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Table 1: Physicochemical characteristics of *Cassia angustifolia*

Solvent	Initial Weight of the Powder (g)	Final Weight of the Powder (g)	Weight of the Crude Extract (g)	Crude Extract %	Colour of the Extract
Hexane	100	95.5	4.5	4.5	Dark Brown
Acetone	100	91.1	8.9	8.9	Dark Green
Methanol	100	86.5	13.5	13.5	Dark Green
Water	100	88.4	11.6	11.6	Dark Red

Table 2: Comparative Analysis of Phytochemical Analysis of Whole Arial Part Extracts of *Cassia angustifolia Cassia angustifolia*

S.NO	TESTS	Hexane Extract	Acetone Extract	Methanol extract	Water extract
01.	Alkaloids				
	Mayer's Test	Negative	Positive	Positive	Positive
	Wagner's Test	Negative	Positive	Negative	Positive
	Dragendroff's Test	Negative	Positive	Positive	Positive
	Hager's Test	Positive	Positive	Negative	Positive
02.	Phenolics				
	$Fecl_2$ Test	Negative	Positive	Positive	Positive
03.	Flavanoids				
	Lead Acetate Test	Negative	Positive	Positive	Positive
	NaOH Test	Positive	Positive	Positive	Negative
	Anthraquinones Test				
	Borntrager's Test	Negative	Negative	Negative	Negative
04.	PhytoSterols				
	Salkowski's Test	Positive	Positive	Positive	Positive
05.	Tannins				
	$Fecl_2$ Test	Negative	Positive	Positive	Positive
	Lead acetate Test	Negative	Positive	Positive	Positive
	Pot. dichromate Test	Negative	Positive	Positive	Positive
	Saponins				
	Froth Test	Negative	Negative	Positive	Negative
06.	Anthocyanins				
	Ammonia-HCl Test	Negative	Negative	Negative	Negative
07.	Leuco- Anthocyanin				
	Iso Amyl Alcohol Test	Negative	Negative	Negative	Negative
08.	Coumarins				
	NaOH Test	Negative	Negative	Negative	Negative
09.	Reducing Sugars				
	Fehling's Test	Positive	Positive	Positive	Positive
	Keller-Kiliani Test	Positive	Positive	Positive	Positive

Table: 3: Total phenol content of *Cassia angustifolia*

concentration of extracts (µg/ml)	% of Phenol content µg GAE/µg			
	HEXANE Extract	ACETONE Extract	METHANOL Extract	WATER Extract
100	13.65	15.10	18.90	16.10
200	21.10	23.10	25.80	23.90
300	28.30	30.30	36.10	32.70
400	35.10	37.60	42.40	39.40
500	42.60	44.80	52.10	47.60

absence of various secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones using standard procedures

Detection of Alkaloids

Extract was dissolved individually in dilute Hydrochloric acid and the resultant solution was clarified by filtration.

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

Table 4: Total Flavonoid content of *Cassia angustifolia*

concentration of extracts (µg/ml)	% of Flavonoid content µg Rutin/µg HEXANE Extract	ACETONE Extract	METHANOL Extract	WATER Extract
100	0.00	8.12	9.80	2.58
200	0.00	12.10	14.36	5.80
300	0.00	19.40	21.64	10.24
400	2.10	24.30	26.70	15.80
500	2.30	30.24	34.58	18.46

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

Dragendroff's Test: Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrate was treated with Hager's reagent (saturated Picric Acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

Detection of Phenols

Ferric Chloride Test: The filtered solution of extract was treated with three drops of freshly prepared 1% Ferric Chloride and Potassium Ferro cyanide. Formation of bluish- green colour is taken as positive.

Detection of Flavonoids

*Alkaline Reagent Test:*The Extract was treated with few drops of Sodium Hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids.

*Lead Acetate Test:*The Extract was treated with few drops of Lead Acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

Detection of Anthraquinones

Free Anthraquinones Test (Borntrager's test): The extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of Benzene, filtered and 5 ml of 10% Ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicates the presence of free anthraquinones.

Detection of Phytosterols

Salkowski's Test: The extract was dissolved in 2 ml Chloroform in a test tube. Concentrated Sulfuric Acid was carefully added unto the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicates the presence of a steroid ring.

Detection of Terpenoids

The extract was added to 2 ml of Acetic Anhydride and Concentrated H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids.

Detection of Tannins

Ferric Chloride Test: The extract was dissolved in water and the resultant solution was clarified by filtration to which 10 % Ferric Chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

Lead Acetate Test: The extract was dissolved in water and to that 10 % Lead Acetate solution was added. Appearance of yellow precipitate confirms presence of tannins.

Potassium Dichromate Test: The extract was dissolved in water and to it a strong potassium dichromate solution was added. Yellow colour precipitate indicates presence of tannins and phenolic compounds.

Detection of Saponins

Froth Test: Extract was diluted with distilled water to 20 ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of "honey comb" froth indicates the presence of saponins.

Detection of Anthocyanins

The extract was added to 2 ml of 2 N HCl and Ammonia. Initial appearance of pink-red colour turning into blue-violet indicates the presence of anthocyanins.

Detection of Leucoanthocyanins

The extract was added to 5 ml of Isoamyl Alcohol. Appearance of red upper layer colour indicates for presence of leucoanthocyanins.

Detection of Coumarins

Three (3) ml of 10% NaOH was added to the extract. Formation of yellow colour indicates the presence of coumarins

Detection of Reducing Sugars

Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for presence of carbohydrates.

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

*Keller Kiliani test (for deoxy sugars in cardiac glycosides):*Fifty **(**50) mg of each extract was dissolved in 2 ml chloroform. H_2SO_4 was added to form a layer and presence of colour at interphase was noted. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

Quantitative analysis of Phenols and Flavonoids

The quantity refers to the intrinsic value of the drug *i.e.*, the amount of medicinal principles present. The active constituents were glycosides, tannins, flavonoids, phenolic compounds, alkaloids, proteins and vitamins. The biological activity of a plant was influenced by the presence of various phytoconstituents. Natural antioxidants such as Vitamin C and Vitamin E directly influence the activity. Certain phytoconstituents such as phenols, flavonoids, tannins, carbohydrates, proteins, Vitamin C and Vitamin E were known to act synergistically. Hence, they have to be quantified in the plant extract.

Determination of total phenol content

The amount of total phenol content, in different solvent extracts of *Cassia angustifolia* was determined by Folin-Ciocalteu's reagent method[10], 0.5 ml of extract and 0.1 ml (0.5 N) Folin-Ciocalteu's reagent was mixed and the mixturewas incubated at room temperature for 15 min. Then 2.5ml saturated sodium carbonate solution was added andfurther incubated for 30 min at room temperature and theabsorbance was measured at 760 nm. Gallic acid was

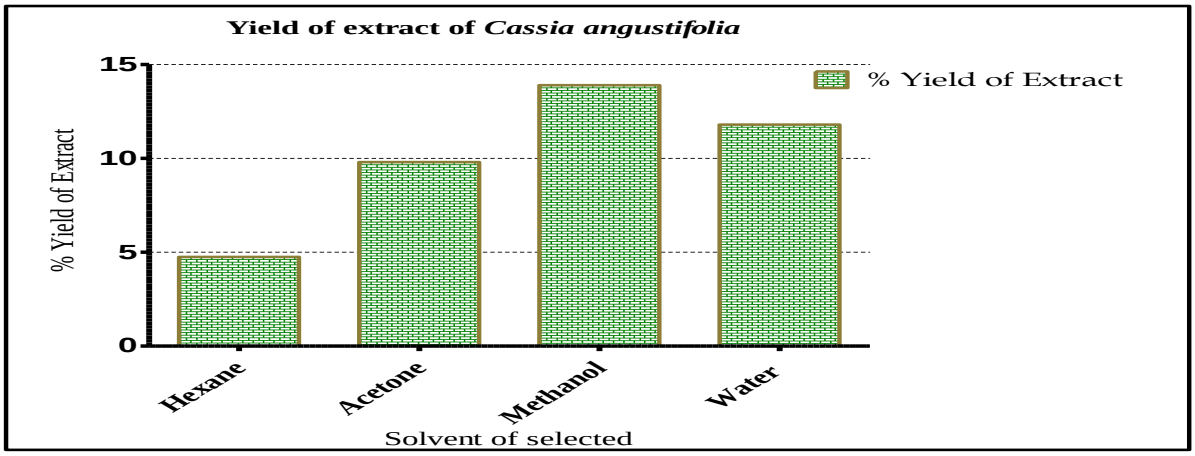
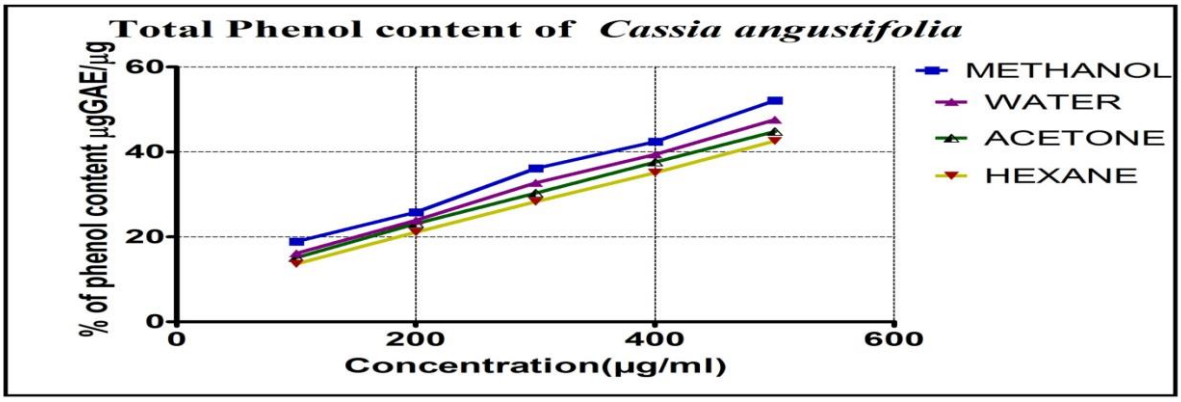


Figure 1: **Physicochemical characteristics of *Cassia angustifolia***



Figuer 2: **Total phenol content of *Cassia angustifolia***

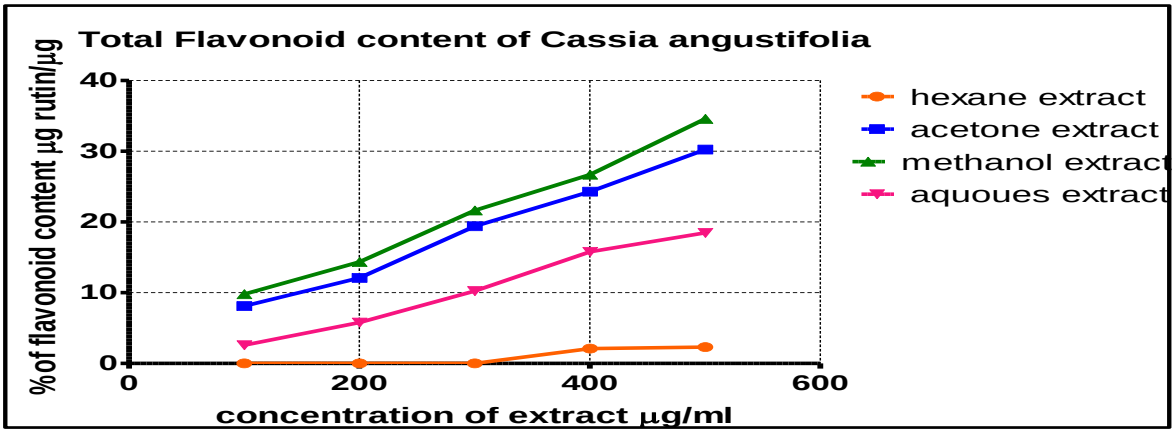


Figure **3: Total Flavonoid content of *Cassia angustifolia***

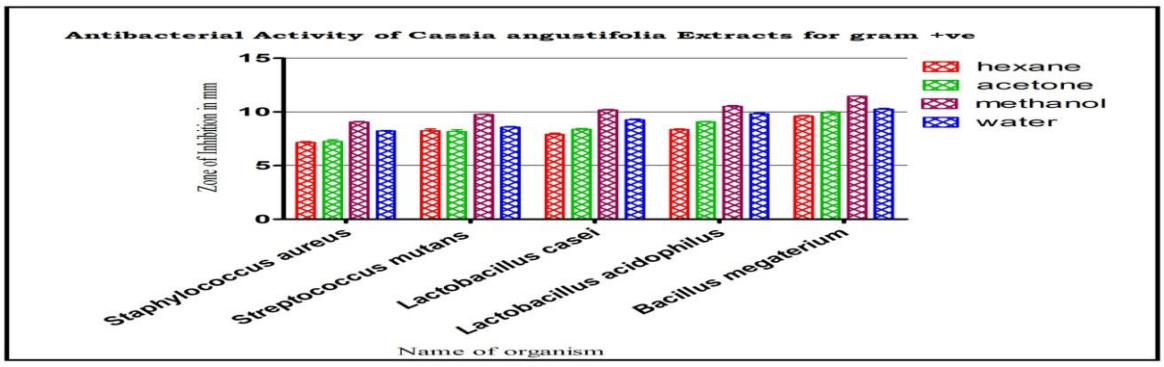


Figure 4: Antimicrobial activity of *Cassia angustifolia* extracts for gram positive

used as a positive control. Total phenol values are expressed in termsof Gallic acid equivalent (mg/g of extracted compounds).

Determination of total flavonoid content

The amount of flavonoid content in different solvent extracts of *Cassia angustifolia* was determination by aluminium chloride colorimetric method[11]. The reaction mixture 3 ml consistedof 1 ml of sample (1 mg/ml) and 0.5 ml of (1.2%)aluminium chloride and 0.5 ml (120 mM) potassium acetate was incubated at room temperature for 30 min. The absorbance ofall samples was measured at 415 nm. Rutin was used as positive control. The flavonoid content is expressed in terms of rutin equivalent (mg/g of extracted compound).

Antimicrobial Study

Antimicrobial activity is expressed as zone of inhibition in millimeters, which is measured with a zone reader. The Hexane, Acetone, Methanol and Aqueous extracts of *Cassia angustifolia* were screened for antimicrobial activity against a wide spectrum of microorganisms and the activity of extracts was compared with appropriate reference standards (Streptomycin for both gram positive and gram negative organisms and fluconazolefor fungal strains). Microorganisms were grown in nutrient agar medium. Dimethyl Sulphoxide and distilled water were used as control.

Test organisms

The microorganisms used for the experiments were procured from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh.

Gram-positive organisms

Staphylococcus aureus(MTCC 3160)
Streptococcus mutans(MTCC 497)
Lactobacillus casei(MTCC 1423)
Lactobacillus acidophilus (MTCC495)
Bacillus megaterium(NCIM 2187)

Gram-negative organisms

Enterococcus faecalis(MTCC439)
Xanthomonas campestris(MTCC2286)
Escherichia coli(ATCC35218)
Pseudomonas aeruginosa (ATCC 9027)

Fungal strains

Candida albicans(ATCC 10231)
Aspergillus niger(ATCC 1015)
Rhizopus oryzae(MTCC 262)
Candida rugosa(ATCC 96275)

Antimicrobial activity of selected medicinal plants[12].

In vitro testing of the sensitivity bacterial and fungal isolates to antimicrobial agents using the disc diffusion assay, according to the guidelines set by the National Committee for Clinical Laboratories Standards (NCCLS, 1997). Antimicrobial activity was screened by agar well diffusion method[12]. The extracts were tested for antimicrobial activity against and grampositive, gramnegative bacteria and fungi.

The Hexane, Acetone, Methanol and Aqueous extracts of *Cassia angustifolia* were prepared separately at different concentrations such as 100µg/ml, 200µg/ml 300µg/ml, 400µg/ml and 500 µg/ml by using Dimethyl Sulphoxide as solvent (DMSO). Streptomycin (2µg/ml) and fluconazole (10µg/ml) were used as positive control (standard) for bacteria and fungi respectively. DMSO was used as negative control. Accurately measured (0.05 ml) solution of each concentration and reference standards were added to the cups with a micropipette.

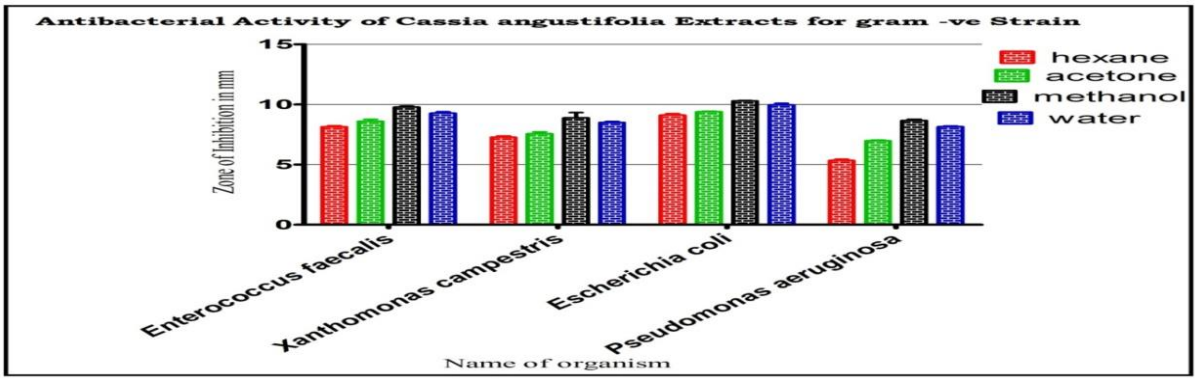


Figure 5: Antimicrobial activity of *Cassia angustifolia* extracts for gram negative

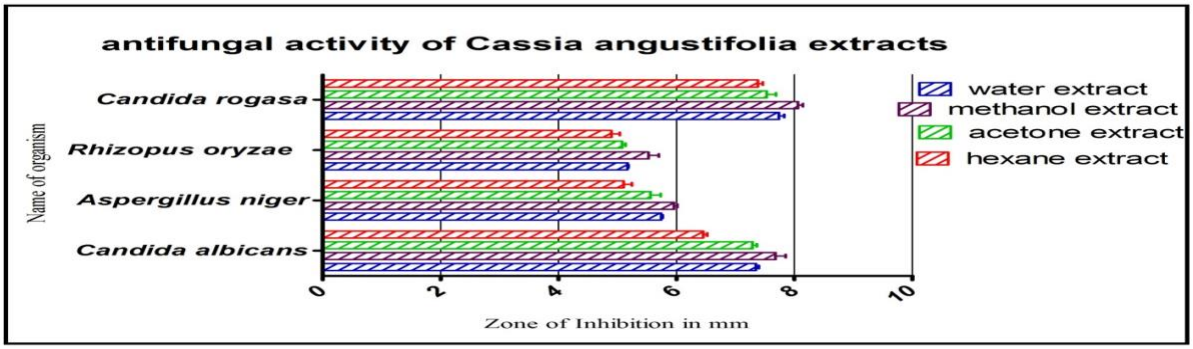


Figure 6: Anti-fungal Activity of *Cassia angustifolia*

All the plates were kept in a refrigerator at 2 to 8°C for a period of 2 hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 °C for 24 hours. The presence of definite zone of inhibition of any size around the cup indicated antibacterial activity.

The solvent control was run simultaneously to assess the activity of Dimethyl Sulphoxide and water which were used as a vehicle. The experiments were performed three times. The diameter of the zone of inhibition was measured and recorded.

RESULTS AND DISCUSSION

The physicochemical characteristics of the *Cassia angustifolia*, Whole plant extracts showed the preliminary information of such plant extracts. The successive extracts of plant material with hexane, acetone methanol and water extract resultants were tabulated in table No: 1. Variation in the colour of the extracts showed the variability of the presence compounds in the solvent extracts, and it also proved that variation in the dissolution of bioactive compounds from non polar to polar solvents. The tabulated results and graphical representation showed (Fig: 1) that yield of extract has been increasing from non polar solvents to the polar solvents[13].

Preliminary screening

The Preliminary qualitative analysis of the extracts showed the initial information of presence/ absence of the various metabolites in the plant extracts.

Qualitative analysis of Cassia angustifolia

Phytochemical screening results of hexane, acetone, methanol and water extracts of *Cassia angustifolia* were tabulated in table:2.These results revealed that presence of phenolics, steroids, alkaloids, flavanoids and reducing sugars in all the extracts of *Cassia angustifolia.* Polar solvent extracts (acetone, methanol and water extracts) of *Cassia angustifolia* showed that occurrence of tannins. Other metabolites like anthraquinones, saponins, anthocyanins, leuco- anthocyanins and coumarins are completely absent in both nonploar and polar extracts of *Cassia angustifolia.*

Total Phenol Content of selected plants

The total phenol content of the *Cassia angustifolia* extracts were determined by folin-ciocalteu method where Gallic acid was used as a standard control[14].The quantitative analysis results of above said extracts were recorded and tabulated in table: 3.These results showed that methanol extract of the *Cassia angustifolia* possess high amount of phenols followed by water, acetone extracts and hexane extract. The graphical representation also confirmed that hexane extracts contain less amount of phenol when compared with polar solvent extracts.

Total phenol content of Cassia angustifolia

Phenolics are the simple, low molecular weight chemical compounds having the acidic hydroxyl group or phenolic group in its structure. Which act in the plant defence mechanisms, determining the type of woods and barks imparting flower colours and flavours. Phenols are the natural antioxidants possessing anti-inflammatory, anticancer, antitumor and hepato protective properties. These antioxidants are natural sources present in vegetables, seeds, plants, barks, peels, leaves and roots [15].

Total Flavonoid content of Cassia angustifolia

In this study the quantification of flavanoids in the extracts of *Cassia angustifolia was* determined by aluminum chloride colorimetric method where rutin was used as a positive control. The results of all these plant extracts were tabulated in table 4 (Fig:3). These results proved that all the polar solvent extracts of *Cassia angustifolia* a significant amount of flavanoids and non-polar solvent extract possess minor amount of flavonoids. Flavanoids were higher in methanol extract as compared to other three extracts. Polar solvent extracts showed dose dependent activity i.e. by increasing the concentration, the amount of flavanoids increased gradually. Aqueous extract contained significantly more amount of flavanoids as compared to acetone and hexane extracts.

Flavonoids are the largest group of polyphenolic compounds having benzo-γ-pyrone structure and ubiquitous in plants.Presently, extensive research is being carried out to find out flavonoids in plant kingdom. These are directly involved in the human dietary habituates and health, hence it is logical to evaluate its functional and structural relationship.Flavonoids have protective activities on humans which include coronary heart disease prevention, anti-inflammatory, antioxidant activity, hepato protective and anticancer activities. Flavonoids are being produced in bulk in the pharmaceutical industry with the aid of microbial biotechnology. Nonetheless,plants also contain useful and efficient quantities of flavonoids which can be used for betterment of human health[16].

Antimicrobial Activity

Antibacterial Activity on gram positive strains

The antimicrobial activity of *Cassia angustifolia* extracts were tested against five gram positive bacteria- *Staphylococcus aureus, Streptococcus mutans, Lactobacillus casei, Lactobacillus acidophilus, and Bacillus megaterium.* After proper incubation the results were recorded and represented in Fig:4. These recorded results said that methanol extract has high antimicrobial activity. These studies also proved that *Bacillus megaterium* was more sensitive to all the plant extracts followed by *Lactobacillus acidophilus, Lactobacillus casei, Streptococcus mutan and Staphylococcus aureus.*

Antimicrobial activity of Cassia angustifolia extracts for gram positive

Antimicrobial activity of the above plant extracts against gram positive organisms also proved that methanol is the most effective solvent for extracting broad spectrum of antimicrobial compounds from plant origin. Water and acetone extracts also showed the moderate antimicrobial activity, but hexane extract showed lesser activity. The inhibitory zones of different extracts varied with the type of microorganism involved in the work.

Antibacterial Activity on gram -ve strains

Most of the pathogenic bacteria belong to gram negative, they causes several diseases like sexually transmitted diseases, respiratory diseases, gastrointestinal problems, nosocomial infections etc. Owing to these problems,

researches show more interest to isolate potential drugs against gram negative organism from plant origin

In the present study, four gram negative pathogenic microorganisms were selected and tested against four extracts of *Cassia angustifolia*. After proper incubation the diameter of inhibition zone was measured and results were recorded and represented in Fig:5.Based on comparative analysis, the methanol extract of *Cassia angustifolia* showed better activity than the other extracts against above said gram negative organisms, followed by water, acetone and hexane extracts.

These studies may helpful for the further isolation of antimicrobial drugs against gram negative organisms. The results of our research also highlight the fact that the methanol solvent extracts exhibited greater antimicrobial activity. So the present observation suggests that the methanol solvent extraction was suitable to verify the antimicrobial properties of medicinal plants which are also supported by many other investigators[17-20]

Antimicrobial activity of Cassia angustifolia extracts for gram negative

Anti fungal Activity

In the present study all the extracts of *Cassia angustifolia* showed antifungal activity and the results were recorded and represented inFig 6. From these results it was proved that Methanol extracts of above said plant showed highest antifungal activity, followed by water and acetone. Whereas hexane extract found lesser antifungal activity than other extracts. Out of four extracts methanol extract found to possess highest antifungal activity against *Candia albicans, Aspergillus niger, Rhizopus oryza and Candida rogasa, .*

Anti-fungal Activity of Cassia angustifolia

Out of four fungal species *Candida rogasa* is more sensitive to *Cassia angustifolia* extracts followed by *Candia albicans, Aspergillus niger, and Rhizopus oryza.* The antifungal activity of these plants makes them potential source of antifungal agents and may be of economic importance as source of antifungal natural plant products.

CONCLUSION

The present work concluded that *Cassia angustifolia* has significant secondary metabolites which are responsible for the various pharmacological activities. It also proved that a polar solvent, especially methanol is the most effective solvent to extract the metabolites from the *Cassia angustifolia.* The current results also said that all the extracts posses' detectable amount of phenols and flavonoids and antimicrobial activity against various pathogenic bacteria and fungi. The methanol extract of *Cassia angustifolia* has most effective antimicrobial activity followed by water, acetone and hexane extracts. Further work is needed toimprove plant based drugs from *Cassia angustifolia.*

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