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Analysis of *Cassia Fistula* for the evaluation of medicine and microbial activity in Bahawalpur

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SUMMARY

Different methanol extracts of *Cassia fistula* were used for preliminary qualitative phytochemical analysis, antibacterial, anti-urease and α-Glucosidase activities. All the extracts were found active and showed positive results for qualitative phytochemical substances. The seed and flower showed positive result for all test of protein, reducing sugar, carbohydrates, flavonoids, saponnins, glycosides, terpenoids and amino acids except phenol, glycosides and steroids while Leaves and barks showed positive results for reducing sugar, carbohydrates, glycosides and amino acid. The flowers showed maximum zone of inhibition 20mm against *Klebsiella pneumonia* and minimum zone of inhibition 10mm against Staphylococcus. The leaves showed maximum 18mm against *Klebsiella pneumonia* and minimum 14mm against *Pseudomonas* and *Escherichia coli*. The bark showed maximum 17mm against Staphylococcus and minimum 13mm against *Escherichia coli*. The seed were found little active and showed maximum 15mm as compared with solvent 13.5mm against *Escherichia coli*. While against other pathogen *Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosai* the seed were found inactive. The Inhibition studies of urease enzyme were observed at 0.5 mg/mL concentration. The CFA extract showed maximum urease activity with percentage inhibition of 94.52±0.73, IC_{50}18.15±0.39 µg/mL and least inhibitory activity was observed In CFD with 91.57±0.68, IC_{50} 25.24±0.43 µg/mL, While CFB and CFC respectively showed 93.27±0.47, IC_{50} 26.27±0.25 µg/mL and 92.31±0.58, IC_{50} 37.42±0.29 µg/mL percentage of inhibition. The methanol extract of CFA, CFB and CFC effect on α-Glucosidase showed almost equal percentage of inhibition 87.3±1.6, 87.3±1.7 and 87.2±1.4 respectively at 1.0 mg/mL concentration, while the CFD showed least percentage of inhibition 76.4±1.5 against α-Glucosidase. All the extracts of CFA, CFB, CFC and CFD showed IC_{50} value of 53.6±1.3, 51.8±1.5 and 47.9±1.2 and 184.7±1.2 µg/mL percentage inhibitions respectively. The Acarbose was used as positive control and showed 65.7±1.9 mM, IC_{50} 375.8±1.7 µM percent inhibition activity.

Keywords: Antibacterial, Anti-urease, Inhhibtion, Phytoanalysis

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INTRODUCTION

Plants are naturally occurring organic resources on earth. Various phytochemicals can be acquire through primary and secondary metabolic process (O. and A., 2011). Initially the plants fulfilled only nutrition value of human but laterally they become the essential part of human health (Mustafa *et al.,* 2017). Man has been using plants as herbal medicine since their existence on earth crust. World Health Organization report shows that millions of people on earth are still dependent on plant for their treatment (Arunkumar, 2009). The plants poses great source of antimicrobial activity. Most of the folk medicine drugs which are being used have been isolated from plants (Ablaka *et al.,* 2011). The natural products are still considered reservoir for therapeutic agents (Mahomoodally *et al.,* 2005).

Phytochemicals are source of active substances which are present in plants naturally and are helpful for curing of human disease. There are also many metabolites which are present in plants and are helpful for the treatment of various infectious diseases (Sohni *et al.,* 2017). In developing countries plants are consider cheap source of treatment than modern techniques (Iniaghe *et al.*, 2009). The primary metabolic substances are protein, lipid, nucleic acid and carbohydrates. They are necessary for the development, growth and reproduction of plants. While secondary metabolic substances are alkaloids, flavonoids and terpenoids which have protective response to antagonistic environmental conditions (Barboza *et al.,* 2018)

They are consider non-nutritive substances produced naturally in plants having defense mechanism properties which protect them from diseases and harsh conditions (Veerachari and bopaiah, 2011). Plants are consider now important constituent in allopathic medicine, homeopathy, aromatherapy (Iniaghe *et al.*, 2009). Previous research showed that Pakistan contains about 6000 flowering trees. About 600 plant species are considered medicinally important (Tariq *et al.*, 2018). There are about 4000 constituents which have been listed as phytochemicals on the basis of their structural and functional activities. These are accumulating in every part of the plans but their efficiency depends on the variety, process of synthesized and supplementary intake (Saxena *et al.,* 2013).

Synthetic medicines are considered harmful as they are toxic to human health. Therefore there is need of using less harmful and active substances which are naturally present in plants (Mohan *et al.*, 2017). Population explosion has changed the trend from synthetic medicine to natural resource as herbal medicine being rich source of curative agent for human disease mechanism (Khandelwal *et al.*, 2016). There is no actual data record available for primitive medicines which have been used by mankind history. But the use of many medicines which have been mentioned in the oldest book in the history of mankind named Riged-Veda. They have also discussed the treatment of infection disease and strengthen the human body of Indian, Chinese, Greek, Egyption and Even Roman ancient civilization (Rajagopal *et al.,* 2013).

There are many pathogen diseases which have been treated by human being through natural source with plants and herbal product. These products are directly consumed or their derivatives are used by people provide the opportunity for researcher discovered new medicine (Aneeja *et al.,* 2011). There are many

diseases through worldwide which have been neglected and untreated such as Leishmaniasis, thus there is need of new medicine and their treatment (Sartorelli, 2009).

Antibacterial activity is mainly concerned with the treatment of bacterial infection. The bacteria have the ability to develop drug resistance after several doses thus researchers need to identify new drugs against bacterial disease (Vimalraj *et al.*, 2009). The infectious disease also developed great resistance against regular use of drugs. Scientific research showed that about 70% of pathogen had developed resistance against many antibiotics (Ghosh *et al.,* 2018). It can lead to serious hazard for human being. The developing countries are at threatened to pathogen resistance as it can create adverse effect on immune system and cause allergic symptoms. From past few year the naturally substances and their product have attract the researcher worldwide to investigate new drugs and antibiotic against infectious disease as it possess the ability of great medicinal (Moselhy *et al.,* 2018).

Activity and composition and of every part of the plant through the world is heterogeneous due to their physiographic factor (Khan *et al.,* 2017). The oxidative stress may cause several human diseases including atherosclerosis, coronary heart disease, neurodegenerative disorder and carcinogenic disorder. The oxidation process is also essential in aging processes (Jothy *et al.,* 2011).

The plants are listed to act as the important properties of anthelmintic, antiseptic and antifungal. They are effective against virus and cancer; are used in the treatment of dyspepsia, asthma, leucoderma, bronchitis, dysentery, muscle weakness, piles and skin and peripheral nerve. The bark ashes used for removing hair from the skin (Preeti *et al.*, 2015; Umair *et al.*, 2022). The prominence in contagious disease and ability of bacterial strains against medicine become free influence the scientist to investigate the new curative resources from plants (Rani *et al.,* 2017).

Parasites are major warning to human vigorous. The lack of advanced facilities and synthetic medicine, the developing countries are major threat to hygienic and diseases resistance (*Arya et al.,* 2010). The naturally existing resource poses the greater ability for inhibiting the pathogen than the synthetic materials (Alam *et al.,* 2009). Therefore there is need of development in the field of medicine develop new effective drugs against pathogen, reduce resistance against microorganism and to investigate the alternative source of common drugs (Bhalodia *et al.,* 2011). Sometimes the antibiotic also disturbed the human immune system (Solanki, 2010). Klebsiella belongs to genus *Enterobacteriaceae* and source of hygienic health condition. It is associated with health infectious disease. The infectious cause by E coli is serious cause of septicemias and it affects the gall bladder, menix, operative infection, skin trauma and the damage of lungs (Chandaand Dave, 2009). The *Pseudomonas* is gram negative pathogen bacteria for both animals and plants. The pneumonia is a lungs infectious disease and also affects the respiratory track caused by *Pseudomonas* (Gales *et al.,* 2001). It also increases the wound infection thus causes and mortality rate of patient (Watanabe *et al.,* 2007). The streptococcus is severe pathogen causing many damaged characteristic of epidermis, sore throat, bacteriaemia, swelling and

redness of skin, puerperal fever, brain fever, respiratory disorder and hypothermia. It is also act as host pathogen for many other diseases diabetes, chickenpox and other acute skin ulcer (Lamagani *et al.,* 2008).

Naturally existing antioxidant sources are considers easier source than synthetic. The plants and fruits are considers rich source of cheaper antioxidant in nature. The oxidative stress is considers main reason behind hazardous disease. It increase the chances of cancer and coronary heart disease. Food poisoning now a day considers massive issue in developing countries is one of them. The antioxidant properties now a day consider essential component in food preservation and anticipation of disease (Iqbal *et al.,* 2006).

There are more than 8000 compound to be known as flavonoids such asmyricetin, rutin, silybin, eriodictyol, apigenin, luteolin, pelargonidin, peonidin, cyanidingand genistein. Phenolic compound are important component in terrestrial and aquatic plants and animals and are efficient antioxidant. The choloroplast is a pigment found in plants, fungi, algae and cyanobacteria which is great compound of carotenoids (Sunkireddy *et al.,* 2013). The carotenoid is important constituent of photosynthesis and blood tissue and play role in many phytochemicals process. They have protective role against cardiovascular disorder, neurological and eyes infection (Fiedor and Květoslava, 2014). The legumes plants are listed to be exhibit good amount of tocopherols, vitamin C and carotenoids antioxidant, which are known to be good source of naturally antioxidant. They are considered essential in our diets and maintenance of our health (Iqbal *et al*., 2004).

Cassia fistula is also identified as Golden showers plant due to presence of yellow flowers. It belongs to leguminous family Fabaceae. It is native to south Asian countries, Pakistan, India, Myammar and Sri Lanka. It is fast growing deciduous tree with a 10-20m height. The leaves are pinnate, 15-60cm long with 3-8 leaflets. The fruits are 30-60cm long which contain several small seeds (Govindrajan, 2009).

Indiantraditional system Aayurvedic indicates that Cassia species have been used against various skin diseases ringworm, eczema and scabies. The leaves and seeds are also being used against much other diseases such as leprosy, flatulence, abdomen pain, dyspepsia, constipation, cough, bronchitis and cardiac disorders (Singh *et al.*, 2013). The previous research paper showed that *Cassia fistula* flower contains the properties of anthraquinone, oxyanthraquinone, tannin, volatile oils and rhein (Antonisamy, 2017). The plant contained fiber and mucilage content which can be used in hypercholesterolemia treatment (Bahorun *et al.,* 2005). It is considers as economic plant as it can also be used as ornamental and for water purification purpose (Hanif *et al.,* 2007). The urease is catalytic proteinases enzyme having a specific function found naturally in plants, animals and fungi. The hydrolysis of Urease produces ammonia and carbon dioxide (Gupta *et al.,* 2015). Current research is an extension of previous studies, with following objectives; such as: Evaluate the bioactive compound in different parts of *Cassia fistula*. Explore the medicinal value. Investigate the Antibacterial activity of methanol extract against four different bacteria *Pseudomonas*,

klebsiella, *Escherchia coli*, and *Streptococcus* causing hazard disease in human being and measured their Zone of inhibition.

MATERIALS AND METHOD

STUDY AREA

The research was conducted in The Islamia University of Bahawalpur. Plants material collected from different parts of Bahawalpur region. The identification was confirmed from the plants expert of Department of Forestry, Range & Wildlife Management. The laboratory experiment for Phytochemicals was progressed in Phytochemicals Analysis Lab of "Department of Forestry, Range and Wildlife Management, University college of Agriculture & Environmental Science, The Islamia university of Bahawalpur". The Antibacterial activity was performed in Chemistry Department Lab at The Islamia university of Bahawalpur.

SAMPLE COLLECTION, EXTRACTION AND PROCESSING

"Dried plant material" was used as a supply for secondary metabolites extraction in plants. Fresh plants material was collected from different parts of Bahawalpur region. The plants material was separately washed with running water and rinsed with distilled water to remove dirt and dried in a shade at room temperature. The dried plants material was pulverized in sterilized electric blender, to a fine powder and store in airtight plastic bags at room temperature. The aqueous extract plants material were prepared by soaking in solvent of methanol. After one week soaked material was first filtered through malmal clothes and then pass through filter paper. The filtered material kept for one week under room temperature for evaporation of methanol. After evaporation the viscous plants extracts was stored in small jars for further experiments.

QUALITATIVE PHYTOCHEMICAL ANALYSIS

The plants sample of Cassia fistula methanol were observed for the confirmation of active substances present in it for the evaluation of medicinal and therapeutic values by adopting following methods.

Test for Proteins Millon's Test

Plant extracts were mixed and shake with 2ml of Millon's reagent; the appearance of white precipitate which changed into red colored when heated confirmed the presence of protein.

Ninhydrin Test

When plant extract were mixed and boiled with "2ml of 0.2%" suspension of Ninhydrin; the violet color formed. The formation of violet color confirmed the presence of amino acids and proteins.

Test for carbohydrates

Fehling's test

When "Fehling A" and "B" reagents mixed at equal volume and poured into the test tube. The plant crude extract added in test tube and boiled. A brick red color

formed at the bottom surface of test tube which confirmed the present of reducing sugars.

Benedict's Test

The plant Crude extract when boiled with "2ml of Benedict's reagent", a reddish brown colored formed which confirmed the presence of carbohydrates.

Molisch's Test

When "2ml of Molisch's reagent" mixed with plant crude extracts in test tube and shaken well, then the 2ml of concentrated H_2SO_4 carefully poured into it. A violet ring formed at interphase which confirmed the presence of carbohydrate.

Test for Phenols and Tannins

The "2ml of 2% solution of $FeCl_3$" mixed with plant extract in test tube. A blue-green or black formed which showed the presence of phenols and tannins.

Alkaline Reagent Test

When plant Crude extract mixed with "2ml of 2% solution of NaOH" in test tube and shaken well. An intense yellow coloration appeared which become colorless on addition of few drops of diluted acid which confirmed the presence of flavonoids.

Test for Saponins

When "5ml of distilled water" mixed with plant crude extracts in a test tube and shaken vigorously. The stable foam formed which showed the presence of Saponins.

Test for glycosides

Liebermann's test

The plant extract was mixed with "2ml of chloroform" and "2ml of acetic acid" and the solution cooled in ice. Then the concentrated H_2SO_4 poured into it. A coloration formed violet which turned to blue and then green which confirmed the presence of steroidal nucleus, i.e., glycine portion of glycoside.

Salkowski's Test

The plant crude extract was mixed with 2ml of chloroform in a test tube and "2ml of concentrated H_2SO_4" poured into it carefully and shake well. A reddish brown color formed which confirmed the presence of steroidal ring, i.e., glycone portion of the glycoside.

Keller-kilani Test

The crude extract was mixed with 2ml of glacial acetic acid containing "1-2 drops of 2% solution of $FeCl_3$". The mixture then poured into another test tube containing 2ml of concentrated H_2SO_4. A brown ring formed at the interphase which confirmed the presence of cardiac glycosides.

Test for Steroid

The plant crude extract was mixed with 2ml of chloroform and concentrated H_2SO_4 poured along sidewise. A red color formed in the lower chloroform layer which confirmed the presence of steroids.

Another test was performed by mixing plant crude extract with 2ml of chloroform and then poured 2ml concentrated H_2SO_4 and acetic acid. The formation of a greenish coloration confirmed the presence of steroids.

Test for Terpenoids

The plant crude extract was dissolved in 2ml of chloroform and placed for evaporation. The "2ml of concentrated H_2SO_4" poured into the dry sample and heated for about 2 minutes. A grayish coloration formed which confirmed the presence of terpenoids.

Test for Alkaloids

The plants Crude extract mixed with "2ml of 1% HCl" and heated gently. Mayer's And Wagner's Reagents poured in to the solution. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

DETERMINATION OF ANTIBACTERIAL ACTIVITY

Preparation of Disc

The prepared methanol plants extract was used for antibacterial activity. The "10µg methanol" extract dissolved in "1µl Methanol"s. The mixture centrifuged at "2000 rpm for 10 minutes". The disc was autoclave at "110°C for minutes" in oven. The 24 disc were spread over wire gauge for each quantity. The disc prepared with the help of micro pipet. The prepared disc stored in sterilized bags at 4°C for further procedure.

Preparation bacterial cultural media

For making nutrient medium for bacteria culture take "10gm nutrient broth" dissolved in "1000ml distilled water" in a beaker and close with aluminum foil. The flask then placed in autoclave at 121.3 °C for 20 minutes. After autoclave take 5ml nutrient medium in "5 different falcon tubes" and take of four different bacteria (*Klebcelea*, *E-coli*, *Streptococcus*, *and Pseudomonas*) in different falcon tubes and 5^{th} falcon tube contained no bacteria called controlled medium. The tubes were placed at "36°C" in shaking machine for "24 hours" for culturing bacteria. After 24^{th} hour the growth activity of bacteria was observed.

Preparation of Nutrient Agar media

Take 13gm agar nutrient dissolved in 1000 ml water. The agar nutrient placed in autoclave for 20 minutes at 121.3 °C. Then the autoclaved agar spread on autoclaved petri plates. The bacterial culture was spread with the help of spreader on agar placed in petri plate. The discs were placed on plates for observing the antibacterial activity. After 24 hours the zone of inhibition were measured with the help of scale compared with special antibiotic disc and solvent disc.

RESULTS

The *Cassia fistula* plants extracts of leaves, barks, flower and stem were investigated for the qualitative phytochemical screenings. These extracted constituent possessed many medicinal, physiological and pharmacological

importance properties. The leaves, flowers, seed and bark contained many active substances and used against many human disease as antiseptic or antibiotic. The methanol extracts investigation showed positive result in many constituent in all samples. The Steroids, Saponins and reducing sugar were present in all four samples and showed positive results. While soluble protein, carbohydrates was present in seed and flower but absent in bark and leaves. Phenol was only present in leaves. Alkaloids were present in seed and bark. Cholesterol was present in all except flowers (see Table 1).

The antibacterial activity of four samples Bark (A), Leaves (B), Seed (C) and Flower (D) extracts studied against four human pathogen bacteria *Klebsiella pneumoniae*, *Escherichia coli*, *Streptoccus pneumonia* and *Pseudomonas aeruginosa*. The antibacterial potential of all sample against four bacteria were assessed in term of zone of inhibition.

The flower showed the maximum zone of inhibition against three pathogen bacteria *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*, while against *E. coli* showed minimum zone of inhibition. Then leaves showed maximum zone of inhibition against *Klebsiella*. While bark showed maximum of zone of inhibition against *Klebsiella, Pseudomoas* and *streptococcus* and minimum zone of inhibition against E-coli. The seed showed minimum zone of inhibition against four pathogen bacteria.

The flowers showed maximum zone of inhibition 20mm against *Klebsiella* and 18mm against *Pseudomonas* as compared to solvent and antibiotic 13mm and 14mm. The flower showed optimum zone of inhibition 16mm against *E. coli* as compared with solvent and antibiotic 13.5mm and 24.5mm and minimum zone of inhibition 10 mm against *streptococcus* as compared to solvent and antibiotic disc 17mm and 19.5mm. The leaves showed maximum zone of inhibition 18mm against *Klebsiella* and bark showed maximum 17mm against *streptococcus* as compared to solvent and antibiotic disc 17mm and 19.5mm. The bark showed 16mm zone of inhibition against *Klebsiella* and *psedumonas* as compared to solvent and antibiotic disc 13mm. The seed showed maximum zone of inhibition 15mm against E-coli as compared to solvent and antibiotic 13.5mm and 24.5mm. The seed showed 14mm zone of inhibition against *Klebsiella*, *psedumonas* and *streptococcus* as compared with solvent 13mm, 13mm, 17 mm and antibiotic disc 14mm, 23.5mm and 19.5mm (Table 2).

DISCUSSION

Cassia fistula is known as folk medicinally important plant with character of many secondary metabolites contained in it. It is native to Asia but distributed through the world. The chemical investigation proved that it is medicinal important character plant with the presence of many phytochemicals (Tapwal *et al.,* 2015). The permanents and regular use of medicine against germs produced resistance against pathogen. The methanolic extracts of pods and leaves found active against *Staphylococcus aureus* (Laxmi *et al*., 2015). The discovery of penciling attracted the researcher to discover new antibiotics and developed the new medicine (Raja *et al.,* 2013).

The phytochemical study of *Cassia fistula* leaves and stem bark showed biologically active and contained many phytochemical substances. The antioxidant property depends on the presence of phenolic content (Bargah and Pardeep, 2017). The qualitative screening of methanolic extract of leaves showed the presence of terpenoids, tannins, saponins and alkaloids (Akhtar *et al.,* 2018). The phytochemical screening of Cassia flower exhibit flavonoids, Tannins, Alkaloids and triterpenes (Moteriya *et al.,* 2015).

The reviewed on Cassia fistula showed that it contained the important activities of Bacteria, Anthelmintic, oxidant, larvicidal, tumor, pyretic and parasitic with important pharmacological and pharmocognostic properties. The DPPH investigation had proved that it contained high amount of flavonoids and phenolic content (Irshad *et al.,* 2012).

The anthraquinones was extracted from *Cassia* and test against pathogen. The investigation showed it was active substance against many bacteria and fungi strains (Yadave *et al.,* 2013). The investigation of methanolic extract of Cassia fistula leaves was found biological active and contained Glycosides, Saponnins and Carbohydrates (Ram and Vishu, 2015).

The seed extracts of methanol exhibit alkaloid, phenol and flavonoid (Jayaraman *et al.,* 2014). The methanol crude extracts of leaves raveled the phytochemical properties of alkaloids, saponins, anthraquinones, coumarins, flavonoids, tannins and glycosides. The leaves and legumes methanol crude extract tested against human pathogen bacteria *Klebsiella, E-coli and Staphylococcus aureus*. The extract exhibited maximum zone of inhibition 25mm and minimum 6mm zone of inhibition (Upadhyay *et al.,* 2011). The flowers, leaves and seed extracts was tested against human pathogen bacteria of *Klebsiella*, *E-coli* and *Pseudomonas*. The MIC was between 7-12mm (Kelkar, 1996).

COMPETING INTEREST

We certify that there is no competing interest with any financial organization regarding the material discussed in the manuscript.

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Table 1: Phytochemical analysis of cassia fistula (Leaves, Flowers, and Bark & Seed)

Sr. No.	Test	A	B	C	D
1	Million's test	-	-	+	+
2	Fehling test	+	-	+	
3	Benedicts test	+	+	+	+
4	Mohlisch's test	-	-	+	+
5	Phenol & tannin test	+	-	-	-
6	Alkaline reagent	-	+	+	-
7	Saponins test	+	+	+	+
8	Glycosides test	-	+	+	+
9	Salkowski test	+	+	+	-
10	Killer killani	-	-	+	+
11	Steroids test	+	-	-	-
12	Terpenoids test	-	+	+	
13	Ninhydrine test	+	+	+	

Table 2: Antibacterial activity of Cassia fistula (Flower, Leaves, Seed& Bark)

Klebsiella	**A**	**B**	**C**	**D**	**Standard disc**	**Solvent**
	16mm	18mm	14mm	20mm	14mm	13mm
Pseudomonas	A	B	C	D	Standard disc	Solvent
	16mm	14mm	14mm	18mm	23.5mm	13mm
Streptococcus	A	B	C	D	Standard disc	Solvent
	17mm	15mm	14mm	10mm	19.5mm	17mm
Escherichia Coli	A	B	C	D	Standard disc	Solvent
	13mm	14mm	15mm	16mm	24.5mm	13.5mm