Influence of Phytochemical Screening and Antibacterial activity on *Cassia auriculata* flowers against some selected Pathogens

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Abstract

Cassia auriculata known as Tanner's Cassia belongs to the subfamily Leguminosae. It is an evergreen shrub grows in many parts of India and Asia. The flower, leaves, stem, root and unripe fruit forms an important part of ayurvedic medicine. It has impressive range of medicinal uses. The present study investigated the phytochemical constituents and the antibacterial activity of *Cassia auriculata* against selected pathogens. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, tannins, glycosides and reducing sugars. The investigation of antibacterial activity revealed the ethanolic extract exhibited more antibacterial activity against the bacteria *Escherichia coli (*21mm). The obtained results revealed that the extract of *Cassia auriculata* was effective against selected pathogens and this study on antibacterial activity provides a support to some traditional medicine. Further research must be needed for effective utilization in a way to discover new drugs.

Keywords: *Cassia auriculata*, antibacterial, phytochemical screening.

Introduction

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Over the centuries, the use of medicinal herbs has become an important part of daily life despite the progress in modern medical and pharmaceutical research. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs (Nascimento *et al*., 2003). Approximately 3000 plant species are known to have medicinal properties in India. In many developing countries, traditional medicine is one of the primary health care system. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, phenolic compounds, steroids, resins, fatty acids etc, which are capable of producing definite physiological action on body (Bishnu *et al*.,2009) Therapeutic efficacy of many indigenous plant for several disorders has been described by practitioners of traditional medicine. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants (Jayashree and Manimegalai, 2008).

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. There are more than thousand known phytochemicals. These phytochemicals act as scavengers of free radicals in the body and protect our body from many type of degenerative diseases and aging (Lee *et al*., 2004).*Cassia auriculata* is a leguminous tree commonly known as avaram, matura tea tree and senna. It occurs in the dry regions of India and Sri Lanka. It is common along the sea coast and the dry zone in Sri Lanka. Its flowers are irregular, bisexual, bright yellow and large. Traditionally, it is used for diabetes, conjunctivitis, joint and muscle pain, constipation, jaundice, liver disease and urinary tract disorders.

Materials and Methods

The flowers selected for the study was *Cassia auriculata*. Fresh flowers were washed thoroughly 2-3 times with running tap water and then with sterile water. Then it was shade dried, powdered and used for extraction.

Test Microorganisms

Human pathogenic bacteria such as *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, and Proteus mirabilis* were collected from Scudder Diagnostic centre, Nagercoil. All the test bacterial species were maintained on nutrient agar media.

Preparation of aqueous flower extracts

The flowers (500g) were washed and the petals of the flowers were removed and shade dried. The powder of flowers were macerated separately with 25ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000rpm for 15min at room temperature. Supernatant was filtered

through Whatmann No.1 filter paper and heat sterilized at 120 C for 30 min. The extract was preserved aseptically in a brown bottle at 4 C until further use (Sukanya *et al*., 2009).

Preparation of solvent extractions

The flowers were washed with clean water. The petals of the flowers were removed and air dried for 5 days. The dried flowers were stored in sealed and labelled containers for use. 20gm of the dried flowers powder were suspended in 120ml of 98% ethanol and left for 2 hours. Therefore, the suspensions were filtered into sterile containers separately using Whatmann No.1 filter paper. The extracts were allowed to dry at a temperature of 40 C into powder. The powder of the extracts obtained were stored in sealed bottles and kept in a refrigerator at 4˚ C until further use as per the method followed by (Akerele *et al*., 2008).

Anti-bacterial activity assay

Antibacterial activity of aqueous and the solvent extracts(ethanol, ethyl acetate, chloroform, acetone and diethyl ether)were determined by disc diffusion method on nutrient agar medium (Anonymous, 1996). Sterile Whatmann filter discs (6mm diameter) were obtained from Scudder diagnostic centre, Nagercoil and inoculums containing bacteria suspension. Then 0.1µ1 each of all aqueous and solvent extracts were placed in the discs made in inoculated plates. The plates were incubated for 24h at 30 ℃ and zone of inhibition if any around the discs were measured in mm. Each treatment consisted of three replicates.

Phytochemical analysis

Preliminary phytochemical tests for the identification of glycosides, steroids, terpenoids, saponins, alkaloids, flavonoids and phenols were carried out for all the extracts by the methods described by Harborne (1973).

Test for alkaloids

Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produced white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui and Ali, 1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent (Evans, 2002).

Test for flavonoids

Five ml of 1% hydrochloric acid extract were shaken with sodium hydroxide, a yellow colour appeared indicating the presence of compound flavonoids (Brown *et al*., 2001).

Test for phenols

To 2ml of test solution, alcohol and then few drops of neutral ferric chloride solution was added and boiled (Harborne, 1973).

Test for saponins

To 2ml of test solution, added 2ml of water and shaked well (Harborne, 1973).

Test for steroids

Four milligrams of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly green bluish colour was observed for steroids (Siddiqui and Ali, 1997).

Test for terpenoids

Four milligrams of extract was treated with 0.5ml of acetic anhydride and 0.5ml of chloroform. Then concentrated solution of sulphuric acid was added slowly and red violet colour was observed for terpenoid (Siddiqui and Ali, 1997).

Test for tannins

To 0.5ml of extract solution 1ml of water and 1-2 drops of ferric chloride solution was added. Blue and green black color showed the presence of tannins (Iyengar, 1995).

Test for glycosides

Glycosides are compounds which upon hydrolysis give rise to one or more sugars and a compound which is not a sugar. To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid were added, and observed for a reddish brown colouration at the junction of two layers and the bluish green colour in the upper layer (Siddiqui and Ali, 1997).

Test for reducing sugars

Extract was shaken with distilled water and filtered. Filtrate was boiled with Fehling's solution A and B for 10 min. Orange and red precipitate indicated the presence of reducing sugar.

Results and Discussion

The phytochemical and antibacterial screening was conducted on four polar and two non polar solvents such as acetone, chloroform, ethyl acetate, diethyl ether, ethanol and aqueous extract of *Cassia auriculata. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhi, Proteus mirabilis* were the selected gram negative bacterial pathogens for the study. The phytochemical analysis of *Cassia auriculata* is shown in (Table: 1; Fig: 1). The maximum number of compounds (8) were noticed in the chloroform extract. The chloroform extract revealed the presence of phytochemicals such as alkaloids, flavonoids, phenols, saponins, terpenoids, tannins, glycosides and reducing sugars. The minimum numbers of phytochemicals (6) were observed in acetone and ethanol extracts. The phytochemical constituents present in the plants contribute definite pharmacological action on the human body (Akinmoladun *et al.,* 2007).

When compared with all the floral extracts the standard control showed maximum zone of inhibition. Except the standard control the ethanolic extract showed remarkable antibacterial activity. The highest zone of inhibition (21mm) were noticed against *Escherichia coli* in ethanol extract. In acetone extract, 19mm zone were recorded against *Escherichia coli* and 18mm against *Klebsiella pneumoniae*. The lowest zone of inhibition (7mm) were noticed in diethyl ether extract and no more inhibitory effect was revealed in aqueous extracts (Table-2; Figure-2; Plate-1). These plants are known to contain various active principle of therapeutic

value and to possess biological activity against a number of diseases (Anushia *et al*., 2009). Earlier researchers identified tannins, phenols, flavonoids, saponins, terpenoids and glycosides in the extracts of selected flowers (Adline and Devi, 2014). Previous research findings also reported the preparation of dried flower extracts and its application against microbial pathogens (Marimuthu *et al*., 2014; Sumitha *et al*., 2015). Higher resolving strength of ethanol in regards to its yield percentage consequently enables it to resolve comparatively more bioactive compounds which might explain the considerable antimicrobial activity compared to the other solvents (Abdullah *et al*., 2013).

Table 1: Phytochemical screening of *Cassia auriculata* using different solvent extracts.

	SOLVENT EXTRACTS					
Phytochemicals	**Acetone**	**Chloroform**	**Ethyl acetate**	**Diethyl ether**	**Ethanol**	**Aqueous**
Alkaloids	+ +	+ + +	++ +	+++	+ +	+
Flavonoids	+++	+++	+++	++	+++	+
Phenols	+++	+ ++	+ +	+ +	+ ++	+++
Saponins	+	+ + +	+++	+ ++	+ + +	++
Steroids	-	-	-	-	-	-
Terpenoids	+++	+++	++	++	+++	++
Tannins	+++	+ ++	+ + +	+ ++	+ +	+ +
Glycosides	-	+ + +	+ +	+ +	-	+++
Reducing Sugars	+++	++	+++	++	-	++

Figure 1: Phytochemical screening of *Cassia auriculata* using different solvent extracts.

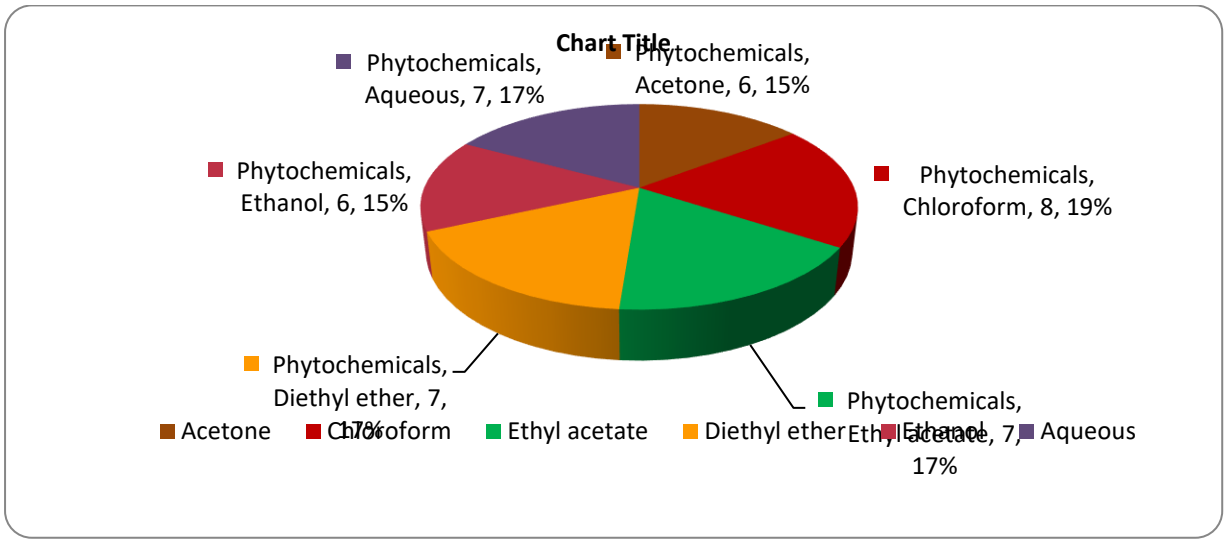


Table 2: Inhibition zone exhibited by Different solvent extracts of *Cassia auriculata* against some selected pathogens.

Pathogens	Zone of inhibition(mm) in different solvents						
	Acetone	Chloroform	Ethyl acetate	Diethyl ether	Ethanol	Aqueous	Control (Amikacin)
Escherichia coli	19mm	12mm	12mm	7mm	21mm	-	22mm
Klebsiella pneumoniae	18mm	-	14mm	8mm	20mm	-	22mm
Pseudomonas aeruginosa	12mm	10mm	11mm	11mm	16mm	-	18mm
Salmonella typhi	12mm	9mm	10mm	12mm	17mm	-	27mm
Proteus mirabilis	15mm	11mm	12mm	17mm	12mm	-	25mm

Fig 2: Inhibition zone exhibited by Different solvent extracts of *Cassia auriculata* against some selected pathogens.

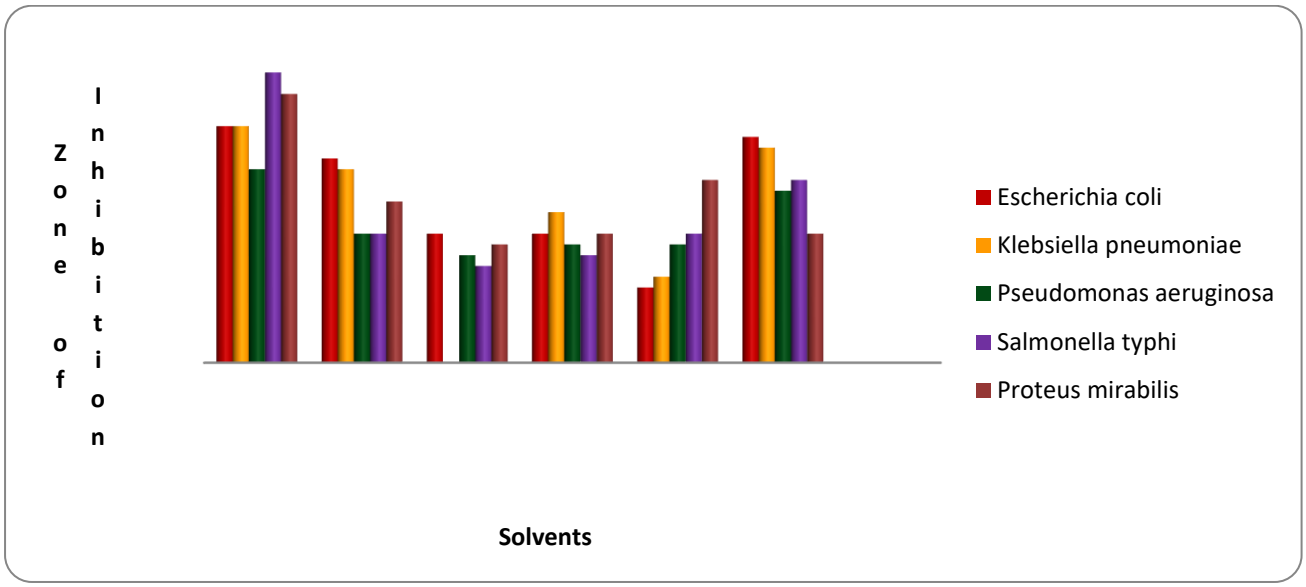
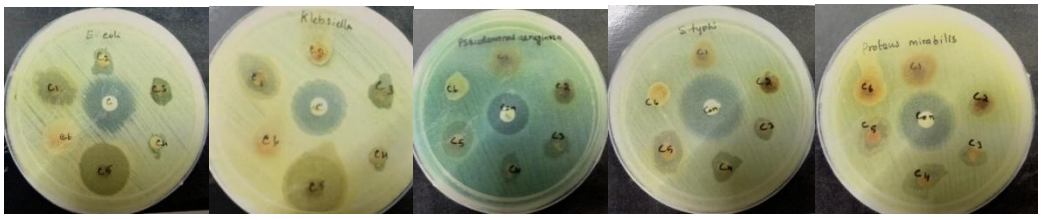


Plate 1: Antibacterial screening of *Cassia auriculata* against selected pathogens with different polar and non polar solvents.



E.coli Klebsiella pneumoniae Pseudomonas aeruginosa Salmonella typhi Proteus mirabilis

Summary and Conclusion

The present study revealed that the tested plant *Cassia auriculata* extracts possessed significant antibacterial activity against selected pathogens. The Maximum number of phytochemical compounds(8) recorded in chloroform extract were alkaloids, flavonoids, phenols, saponins, terpenoids, tannins , glycosides and reducing sugars. The ethanolic extract of *Cassia auriculata* showed the maximum inhibition zone of 21mm against *Escherichia coli.* These wide range of antibacterial activity of *Cassia auriculata* can be used and administered in the ethno medicine practice. It can be a source of novel useful drugs and of greater pharmacological importance. Further studies are needed with this plant to isolate, characterize and elucidate the structure of the bioactive compounds for industrial drug formulation.

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