Jundishapur Journal of Microbiology
Published online 2022 April

Research Article
Vol. 15, No.1 (2022)

Comparative Phytochemical Screening and Antibacterial Activity of *Azadirachta Indica* and *Cassia Auriculata*.

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Received 2022 February 2**; Revised** 2022 March 20**; Accepted** 2022 April 24

ABSTRACT:

The present study of phytochemical screening and antibacterial activity done to identify potential drugs. The flower extracts of *Azadirachta indica* and *Cassia auriculata* were chosen for a comparative study. It resulted that *Azadirachta indica* flower extract proved to be highly potent against all most of the tested organisms and can treat different aliments

Keywords: Phytochemical, Antibacterial activity, drugs.

INTRODUCTION:

Medicinal plants have been of age long remedies for human diseases because they contain components of therapeutic value. They are rich sources of ecologically developed secondary metabolites, which are potential remedies for different ailments. In many developing countries, traditional medicine is one of the primary health care systems. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action.

Microorganisms harmful to human beings are termed as pathogens. In the recent past, due to the emergence and increase of such pathogenic strains resistant to multiple antibiotics and the continuing emphasis on health care costs, many researchers have tried to develop new, effective antimicrobial reagents free of resistance and cost. The antimicrobial activity is known to be a function of the surface area in contact with the microorganisms. Drug resistance is a serious global problem, and spread of resistance poses additional challenges for clinicians and the pharmaceutical industry. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and emerging infectious diseases (Parekh and Chanda, 2008). The world

around is covered with many plants from which we can extract many antimicrobial compounds which microbes are not exposed to them.

MATERIALS AND METHODS:

The flowers selected for the study was *Azadirachta indica* and *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. 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.

For aqueous extraction, 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). For solvent extractions, dried flowers were stored in sealed and labeled 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˚C 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).

1. 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 neural or slightly acidic solution by Mayer's reagent (Evans, 2002).

2. 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).

3. Test for phenols
To 2ml of test solution, added alcohol and then few drops of neutral ferric chloride solution was added and boiled.

4. Test for saponins
To 2ml of test solution, added 2ml of water and shake well.

5. 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

6. 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.

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

8. 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.

9. 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 precipitates indicates the presence of reducing sugar.

RESULT AND DISCUSSION:

The different extracts of *Azadirachta indica* exhibited highest antibacterial activity against the selected pathogens. The ethanol extract of *Azadirachta indica* showed 23mm zone of inhibition against *Klebsiella pneumoniae*. *Salmonella typhi* exhibited 21mm zone of inhibition in ethanol extract followed by *Escherichia coli* 20mm in the same extract. The diethyl ether extract showed 16mm inhibition zone against *Salmonella typhi*. The pathogens *Pseudomonas aeruginosa* also showed 15mm inhibition in chloroform and ethyl acetate. In acetone extract *Pseudomonas aeruginosa* showed 14mm and *Salmonella typhi* 11mm zone of inhibition of 10mm was noticed against *Pseudomonas aeruginosa* in diethyl ether extract and ethanol extract. The least zone was noticed against *Escherichia coli* and *Proteus mirabilis* in most of the solvents. Aqueous extracts showed no activity against all the pathogens except *Proteus mirabilis* (Table 1.1; Fig 1.1, 2.1). The phytochemical screening revealed the presence of a greater number of phytochemicals (Table 2.1; Fig.2.3). More number of compounds was present in chloroform extracts. Alkaloids, phenols, saponins, steroids, tannins, glycosides and reducing sugars were present in chloroform extracts. Acetone, ethanol and diethyl ether

revealed the presence of alkaloids, phenols, saponins, steroids, tannins and glycosides. Alkaloids, phenols, saponins, terpenoids, tannins and glycosides were present in ethyl acetate and aqueous extract

In *Cassia auriculata* the maximum inhibition zone was recorded in ethanol extract against *Escherichia coli*. (Table 1.2; Fig.1.2, 2.2). The ethanol extract also shows 20mm inhibition zone in *Klebsiella pneumoniae*. In acetone extract 19mm zone was recorded against *Escherichia coli* and 18mm against *Klebsiella pneumoniae* was noticed against *Pseudomonas aeruginosa* (16mm), *Salmonella typhi* (17mm) in ethanol extract and *Proteus mirabilis* (17mm) in diethyl ether extract. The zone of inhibition of 15mm was recorded against *Proteus mirabilis. Pseudomonas aeruginosa, Salmonella typhi* and *Proteus mirabilis* exhibited 10mm-12mm zone against some pathogens. Least activity was noticed in diethyl ether against *Klebsiella pneumoniae* and *Escherichia coli.* The phytochemical study of *Cassia auriculata* noticed the presence of various compounds (Table 2.2; Fig. 2.4). Alkaloids, phenols, flavonoids, saponins, terpenoids, tannins, glycosides and reducing sugars were present in chloroform, ethyl acetate, diethyl ether and aqueous extract. Except steroids and glycosides all the other compounds were present in acetone and ethanol extract.

Azadirachta indica flower extract proved to be highly potent against all most of the tested organisms than *Cassia auriculata*. Francine *et al*., (2015) carried a study on different parts of *Azadirachta indica* and also revealed the fact that ethanol extracts were the best terms of effectiveness against pathogens. Previous findings of other authors also reported similar results in selected flower extracts (Raut *et al*., 2014; Maneemegalai and Naveen, 2010).

Table 1.1 *Azadirachta indica* extracts showing antibacterial activity

Pathogens	**Zone of inhibition (mm) in different solvents**						
	Acetone	**Chloroform**	**Ethyl acetate**	**Diethyl ether**	**Ethanol**	**Aqueous**	**Control (Amikacin)**
Escherichia coli	-	9mm	7mm	-	20mm	-	18mm
Klebsiella pneumoniae	-	8mm	8mm	-	23mm	-	26mm
Pseudomonas aeruginosa	14mm	7mm	11mm	10mm	15mm	-	21mm
Salmonella typhi	11mm	15mm	15mm	16mm	21mm	-	28mm
Proteus mirabilis	-	9mm	7mm	7mm	10mm	8mm	22mm

Table 1.2. *Cassia auriculata* extracts showing antibacterial activity

Pathogens	**Zone of inhibition (mm) in different solvents**						
	Acetone	**Chloroform**	**Ethyl acetate**	**Diethyl ether**	**Ethanol**	**Aqueous**	**Control (Amikacin)**
Escherichia coli	19 mm	12 mm	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

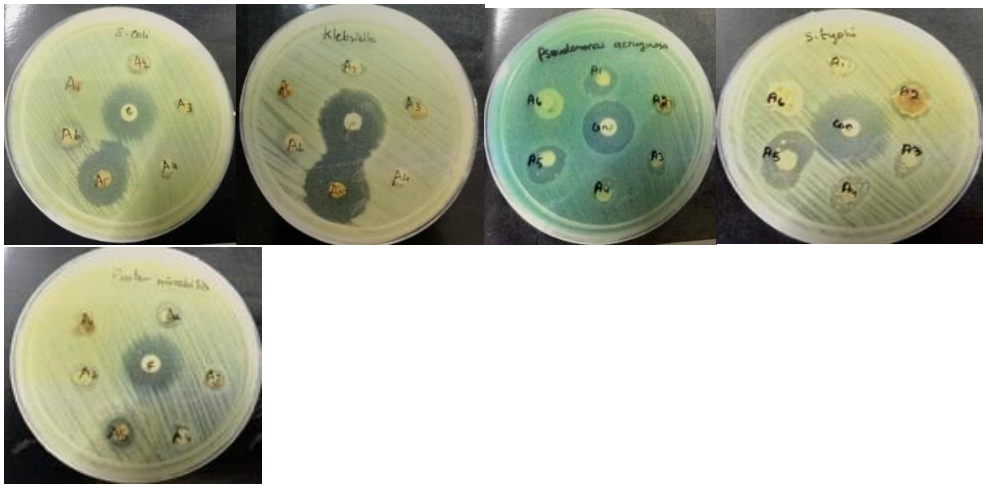

Fig1.1 *Azadirachta indica* extracts showing antibacterial activity

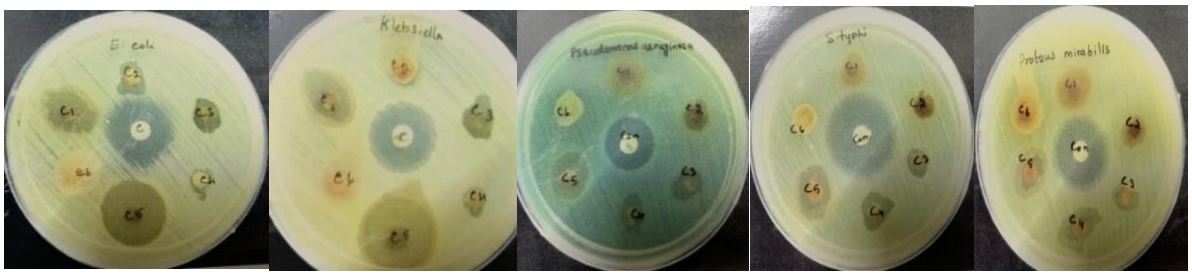

Fig 1.2 *Cassia auriculata* extracts showing antibacterial activity

Table 2.1. Phytochemical analysis of *Azadirachta indica*

Phytochemicals	Solvent extracts					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Diethyl ether	Aqueous
Alkaloids	++	++	++	+	+	+
Flavonoids	-	-	-	-	-	-
Phenols	+	++	++	+	+++	++
Saponins	+	++	+++	+	++	+++
Steroids	+	++	+	-	+	-
Terpenoids	-	-	-	++	-	+
Tannins	+	++	++	++	+++	+
Glycosides	+	+	++	+	+++	+

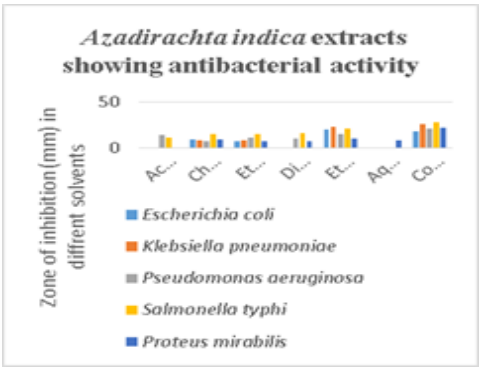

Fig. 2.1

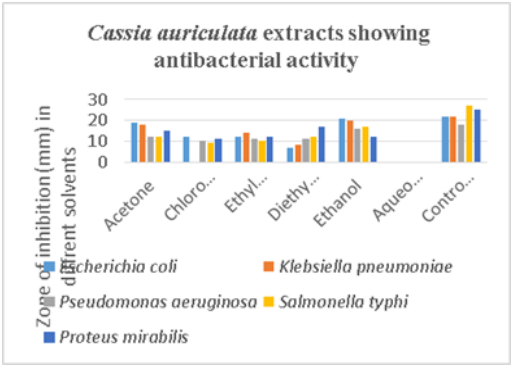

Fig 2.2

Table 2.2. Phytochemical analysis of *Cassia auriculata*

Phytochemicals	Solvent extracts					
	Acetone	Chloroform	Ethanol	Ethyl acetate	Diethyl ether	Aqueous
Alkaloids	++	+++	++	+++	+++	+
Flavonoids	+++	+++	+++	+++	++	+
Phenols	+++	+++	+++	++	++	+++
Saponins	+	+++	+++	+++	+++	++
Steroids	-	-	-	-	-	-
Terpenoids	+++	+++	+++	++	+++	++
Tannins	+++	++	++	+++	+++	++
Glycosides	-	+++	-	+++	++	+++
Reducing Sugar	+++	++	+	+++	+++	++

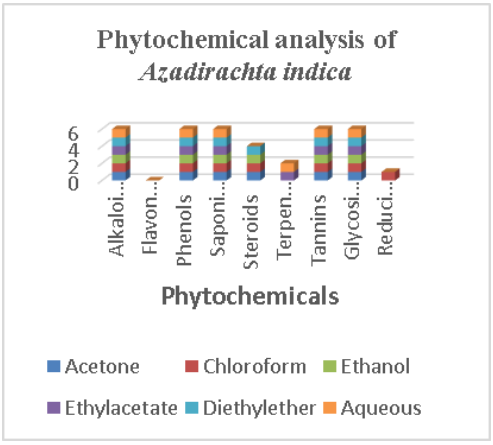

Fig 2.3

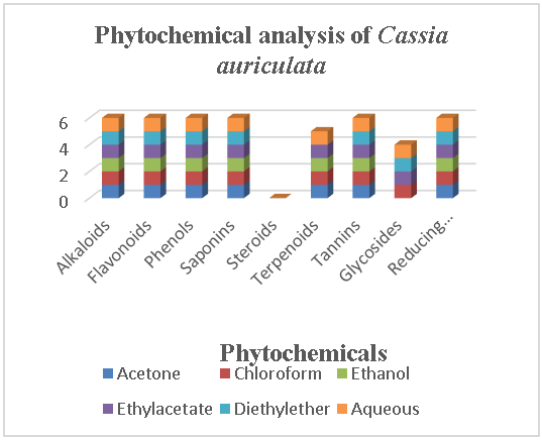

Fig 2.4

CONCLUSION:

The study findings gives the information on the wide range of antibacterial activity of selected flowers can be used and administered in the ethno medicine practice. It can be a source of novel useful drugs and of greater pharmacological importance which can be used to treat various types of infection.

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