Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(11):740-744

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Phytochemical screening and antibacterial evaluation of the leaf, flower and seed coat extracts of *Cassia alata L*

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ABSTRACT

Preliminary studies on the phytochemistry and extract of diethyl ether, chloroform and acetone of Cassia alata L leaf, flower and seed coat were examined for antimicrobial properties. Extract tested against clinical isolated of Pseudomonas sps, Escherichia coli, Staphylococcus sps, Shigella boydii and Salmonella sps. The zone of inhibitions produced by the extracts in disc diffusion assay against the test microorganisms, the produced zones that measured in mm. Preliminary phytochemical analysis showed that the extracts contained tannin, flavonoid, terpenoid, cardiac glycosides, steroids and terpenoids, absence of alkaloids.

Keywords: Antibacterial activity, Phytochemistry, Extraction of *Cassia alata L.*

INTRODUCTION

According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [4]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolic compounds [6]. The general body care and oxidative stress diseases. Ethanobotanical methods provide important leads in the search provide important leads in the search for new principles and templates for more potent and safer drugs and cosmetics discovery. Plant extracts can be potential for high biological activities rather than isolated pure compounds through selective removal of bulk non active components from the extracts by using appropriate extracting solvents and methods. The use of plants for treatment of various ailments dates back to over 5000 years. A great source of ancient information is contained in the 'Vedas' and more specifically 'Yajur Veda' is the main source of such information. In these; 'Vedas' the medicinal importance of many plants has been mentioned. The earliest monumental contribution of 'Ayurveda' is the 'Samhitas' of 'Charak' and sushrut (1000 – 700 B.C), which included 500 plants with their therapeutic prosperities. Herbal medicines represent the most important field's traditional medicine all over the world. To promote the proper use of herbal medicine and determine their potential as source for new drugs it is essential to investigate the medicinal plants which have folklore reputation is a more intensive way [11]. The plant has also been reported to possess several pharmacological properties including anti-inflammatory [15, 2] and analgesic activity [10]. There is, however, limited information regarding the

traditional use of *Cassia alata L* for cancers and there is also limited scientific data available regarding cytotoxic properties of *Cassia alata L* extracts. Thousands of secondary plant products have been identified and it is estimated that the same quantity of these compounds may still exist. Since secondary metabolites from natural resources have been elaborated with in living friendliness than totally synthetic molecules making those good candidates for further drug development [9].

The world is now entering into a biological revolution era to minimize the wide spread loss of bio resources as a consequences of climate change, containing rise of energy consumption. Population pressure, agricultural and degradation and urbanization. The future of millions of people all over the world depends on our ability to conserve our biological wealth and use it intelligently for human welfare. Biotechnology tools available today can be powerful in the judicial management of bio resources. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. The research into plant with use as pain relievers, anti-inflammatory agents, should therefore is viewed as a fruitful and logical research strategy in the search for new analgesic and anti-inflammatory drugs. The genus cassia belongs to the family Leguminosae, which comprises 200 genera and 2500 species, which are distributed throughout the world. This study is aimed at determination the phytochemistry and antimicrobial activity of the different plant parts of *Cassia alata L* against the following microorganisms *Pseudomonas sps, Escherichia coli, Staphylococcus sps, Shigella boydii* and *Salmonella sps.*

EXPERIMENTAL SECTION

Collection of Plant Materials:
The medicinal plant of *Cassia alata L* were collected from natural habitat at kulithalai in karur District. The collected materials were sorted out the leaf, flower and seed coat were than shade dried at room temperature (37^0C) the shade dried materials were grind into powder form, and stored in room temperature.

Micro Organism's Strains
Micro Organism's purchased from K. A. P. Vishwanatham Government Medical College, Trichy-20. Medicinal plants were tested against the bacteria such as *Pseudomonas sps, Escherichia coli, Staphylococcus sps, Shigella boydii* and *Salmonella sps.*

Sterilization of Plant Materials
The disease free and fresh leaves of plant were selected for this investigation. About 2 grams of fresh and healthy leaves were taken. These are washed with tap and distilled water for three times. Then, surface sterilized with 0.1% mercuric chloride for 2 minutes. Again the plant materials were washed thoroughly with distilled water for three times.

Preparation of Plant extracts
The whole plants along with leaf, flower and seed coat dogged out from their carefully. Plant was collected from nearby places, and the leaf and seed were separated and washed under running tap water. Thoroughly washed leaf, flower and seed coat were allowed for shade drying under room temperature in the laboratory. The dried leaves were ground to fine powder using a blender. The Powder was preserved in an air tight bottle for further studies.

Preliminary phytochemical analysis
Cassia alata L diethyl ether, chloroform, acetone, extract of leaf was preliminary qualitatively screened for phytochemicals as per standard biochemical procedure. The crude extract was diluted with diethyl ether, chloroform, acetone to the concentration of 1mg/ml. The qualitative photochemical analysis of crude extract, was performed to determine the presence of tannin, saponin, flavonoid steroid, cardiac glycosides, alkaloids, anthraquinones.

Phytochemical Screening
The samples were crusted into fine powder and dissolved separately in 100ml of solvent. The solution was kept at room temperature for seven days to allow the extraction of compounds from seeds. The solution of each sample was stirred after every 24hrs using sterile glass rod.

After 7 days the Solution was filtered through what man filter paper No-1 and a greenish filtrate was obtained. The solvent was evaporated and sticky substances obtain that was stored in the refrigerator and suspended in 10% dimethyl sulfoxide prior to use. In the present study, antibacterial activity from the crude extract of the leaf, flower

and seed coat of the plant *Cassia alata L*. were evaluated. *Cassia alata L* is medicinally important plant, used in Ayurveda and unani system of medicine. Chemical tests were carried out both on the ethanolic extract and on the powdered specimen using standard procedures to identify the constituents as described by. The specific procedure involved for the evaluation of a particular group of chemical is mention below [7].

Tannins
1ml of water and 1-2 drops of ferric chloride solution were added in 0.5ml of extracted solution, blue colour was observed for Gallic tannins and green black for catecholic tannins [8].

Saponins
Foam test small amount of extract was shaken with little quantity of water it foam produced. Persistent for ten minutes it indicates the presence of saponins [13].

Flavanoids (alkalin reagents test)
4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was added. To this solution, 5-6 drops of concentrated hydrochloric acid was added and red colour was observed for flavonoids and orange colour for flavonoid [14].

Steroids
Two ml of acetic anhydride was added to 0.5g sample extract of each sample with 2 ml H_2SO_4 the colour changed from violet to blue or green in some samples indicating the presence of steroids.

Terpenoids (Salkowski test)
Five ml of each extract was mixed in 2ml of chloroform and concentrated H_2SO_4 3ml was carefully added to form a layer. A reddish brown colouration of the interface was formed to show the presence of terpenoids**.**

Cardiac Glysides (Kellar-Killant test)
5 ml of each extract was treated with 2ml of glacial acetic acid containing drop of ferric chloride solution. This was under layer with 1ml of concentration sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring. While in the acetic acid layer a greenish may from just gradually throughout thin layer.

Alkaloids
The extract of *Cassia alata* was evaporated to dryness and the residue was heated on a boiling water bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent [12]. The samples were then observed for the presence of turbidity or yellow precipitation [5].

Anthraquinone
0.5 g of powdered plant was boiled with 10 ml of ferric chloride (10%) and 5 ml of dilute HCl for 5 mins. The mixture was filtered while hot, cooled and the filtrate was shaken with equal volume of chloroform. The layers were allowed to separate in a separating funnel, the chloroform layer was transferred into another test tube containing 5 ml of 10% ammonia solution and the upper aqueous layer was observed for a bright-pink colour showing the presence of anthraquinones.

Antimicrobial assay of the plant extracts
The antimicrobial used was the standard Agar Disc Diffusion assay adapted from [1, 3]. Mueller Hinton Agar was prepared for the study. Mueller Hinton agar plates were swabbed with a suspension of each bacterial species, using a sterile cotton Swab. Subsequently, the sterilized filter paper discs were completely saturated with the test compound. The impregnated dried were placed on the surface of each inoculated plate. The Plates were incubated overnight at 37^0C. Each organism was tested against pathogenic microorganisms. The test materials having anti microbial activity inhibited the growth of the micro organisms and a clear, halo zone of inhibition was visualized surrounding the disc. The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition in mm.

RESULTS AND DISCUSSION

Preliminary Phytochemical Analysis

The phytochemical analysis of diethyl ether, chloroform, acetone extract of leaf, flower and seed coat extract of *Cassia alata* was analysed for the compounds such as tannin, saponin, flavonoid, steroid, terpenoids, cardiac glycosides, alkaloids, anthraquinones. The preliminary phytochemical analysis revealed the presence of six compounds i.e. tannin, flavonoid, terpenoid , cardiac glycosides, steroids and terpenoids absence of alkaloids. (Table-1, 2, 3)

Antibacterial activity

The antibacterial property of diethyl ether, chloroform, acetone extract of leaf, flower and seed coat extract of *Cassia alata L.* was analysed against bacterial pathogens. Out of these five bacterial pathogens four were found to be gram negative *Echerichia coli, Pseudomonas sps, shigclla boydii, Salmonella sps* and one were gram positive *Staphylococcus sps*. Disc diffusion method was used to evaluate the antibacterial activity of taken samples. After twenty-four hours the minimum inhibitory zone of Diethyl ether leaf, flower and seed coat extract of *Cassia alata L* were measured. The leaf, flower and seed coat extracts of *Cassia alata L.* were found tested for their *shigella boydii* was found to be more susceptible toward the diethyl ether, chloroform and acetone extracts of seed coat with a maximum inhibitory zone (5.5 mm each), followed by chloroform in flower with a maximum inhibitory zone (3.5mm). Chloroform and diethyl ether in the following inhibitory zone of maximum leaf and flower (5.5 mm each), acetone and diethyl ether extracts of flower and leaf with a maximum inhibitory zone (5.6 mm each). *Echerichia coli* were tested in *Cassia alata L.* were found to be acetone and diethyl ether extracts of seed coat with a maximum inhibitory zone (5.3 mm each), followed by acetone (4mm), maximum inhibitory zone in acetone and diethyl ether (3.9 mm), and the chloroform extracts did not show any inhibition against of *Pseudomonas Shigclla boydii* was found to be more susceptible toward the acetone extracts seed coat maximum inhibitory zone (5.5 mm), chloroform and diethyl ether extracts in flower inhibitory zone (3 mm each), acetone, chloroform and diethyl ether extracts seed coat, flower and leaf inhibitory zone (3mm).

Table 1: Preliminary phytochemical analysis of diethyl ether, chloroform, acetone extract of leaf of *cassia alata L*

S.No	Test	Leaf Acetone extract	Leaf Diethyl ether extract	Leaf Chloroform extract
1	Tannins	-	+	-
2	Saponins	-	-	-
3	Flavanoids	+	-	+
4	Steroids	+	-	+
5	Terpenoids	+	+	-
6	Cardiac glylosides	+	+	+
7	Alkaloids	+	+	+
8	Anthraquinonus	-	+	-

Table 2: Preliminary phytochemical analysis of diethyl ether, chloroform, acetone extract of flower of *Cassia alata L*

S.No	Test	Flower Acetone extract	Flower Diethyl ether extract	Flower Chloroform extract
1	Tannins	-	+	-
2	Saponins	+	_	+
3	Flavanoids	+	-	-
4	Steroids	+	+	+
5	Terpenoids	+	+	-
6	Cardiac glylosides	+	-	-
7	Alkaloids	-	-	-
8	Anthraquinonus	+	+	+

Staphylococcus sps was found to be more susceptible toward the acetone and diethyl ether extracts seed coat maximum inhibitory zone (5.6 mm), followed by acetone flower (3.1 mm), inhibitory zone acetone and chloroform leaf and flower (3 mm, 3.5 mm), and chloroform extracts did not show any inhibitory against in *Pseudomonas* at leaf , flower and seed coat.

Salmonella was found to be more susceptible toward the chloroform and diethyl ether extracts of seed coat with a maximum inhibitory zone (5.5 mm each), chloroform in seed coat (2.9 mm), followed by flower (3.1 mm), acetone,

chloroform and diethyl ether extracts of flower, leaf maximum inhibitory zone (4 mm each). The results obtained are encouraging as the acetone, chloroform and diethyl ether extracts have shown considerable antibacterial activity against the tested organisms.

Table 3: Preliminary phytochemical analysis of diethyl ether, chloroform, acetone extract of seed coat of *cassia alata L*

S.No	Test	Seed coat acetone extract	Seed coat Diethyl ether extract	Seed coat chloroform extract
1	Tannins	-	-	-
2	Saponins	-	+	-
3	Flavanoids	+	+	+
4	Steroids	-	-	-
5	Terpenoids	-	+	-
6	Cardiac glylosides	+	+	+
7	Alkaloids	-	-	-
8	Anthraquinonus	+	+	+

Table 3: Antibacterial activity of *Cassia alata L.* plant extract

S. No	Number of organisms	Acetone			Chloroform			Diethyl ether		
		L mm	F mm	SC Mm	L Mm	F mm	SC mm	L mm	F mm	SC Mm
1	*Shigella boydii*	1.9	3.4	3.1	2.0	3.2	2.9	3.9	2.6	5.6
2	*E.coli*	3.2	2.9	3.0	1.5	3.5	2.0	3.0	3.0	5.6
3	*Staphylococcus sps*	3.0	3.1	4.0	3.1	1.1	2.9	4.0	3.1	2.9
4	*Pseudomonas sps*	1.9	1.2	1.7	0.3	0.2	0.3	3.9	3.0	5.5
5	*Salmonella sps*	1.2	2.1	3.2	1.7	1.5	1.9	3.0	2.0	5.3

Symbols: L: Leaf; Symbols: F: Flower ; Symbols: SC: Seed coat

CONCLUSION

The result obtained from the test carried out indicates that *Cassia alata L* can help control disease caused by *Staphylococcus aureus* which is a major pathogen for human infection varying from food poisoning and skin infections to severe life threatening infections such as *staphylococcal aureus, E.coli* and other coli forms which cause urinary tract infection, diarrhea, sepsis and meningitis. Phytochemical analysis revealed the various metabolites present in *Cassia alata L* used. Thus providing knowledge of the metabolites responsible for its therapeutic quality. However, more research has to carried out so as to known longevity of the metabolites in the plant and the effect of low or high concentration usage. Therefore, further pharmacological and clinical studies are required to understand the mechanism and the actual efficacy of these herbal extracts in treating various infections and skin diseases like psoriasis.

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