

Phytochemical Screening and Bioactivity Study of *cassia alata* Leaves

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Cassia alata, a plant noted for its ethanomedical and ornamental importance is identified for our study with the clear objective of isolating broad spectrum of active molecules by solid liquid extraction using solvents of varying polarity like ethanol, chloroform, acetone and petroleum ether. Phytochemical screening of the crude extracts revealed the presence of phenols, flavonoids, alkaloids, proteins, glycosides and terpenoids. A positive correlation was obtained for the presence of phytochemicals and the bioactivity of the plant with relevance to antibacterial, antioxidant and anticancer activity. 20mm zone of inhibition was observed for the chloroform extracts on *Bacillus sp.*, The radical scavenging activity was seen for all the extracts using DPPH and their corresponding IC₅₀ values were calculated. Acetone reduced the free radicals by 82%. The anticancer activity was carried out with the dose response analysis on Hep2 and HCT-15 cell lines and their percentage cytotoxicity was calculated

Key words: Phytochemicals, *Cassia alata*, antibacterial, antioxidant, anticancer.

Human diligently sought the help of nature for all the intricacies of life's progression. Plants have become indispensable company in his day today life as food, fodder, medicine etc., His insight to look for cure had developed way long before he understood their mechanism of actions. He formulated his new findings as natural system of medicine and the information of which was passed onto their generations. The rich biodiversity of phytoconstituents had made him believe that for every disease there is a cure in plants¹. It is evident that even now certain countries in Asia and Africa solely depend on plants for their medicine². Their success as ethanomedically

important plants has drawn the attention of researchers and scientists into the unexplored arena of modern drug discovery. Primary information of the plants are procured from the folk lore practices and here an attempt was initiated to provide scientific evidence of one such plant *Cassia alata*, commonly known as candle brush. The ayurvedic system of medicine recognizes the plant in the treatment of constipation, stomach pain, ringworm and skin disease³. This tropical plant claims a promising cure globally for various states in Ivory Coast and Ghana where people take the decoctions of the bark, roots and leaves for treating gastrointestinal related disorders, asthma bronchitis and urinary tract infections. In Adamawa and Taraba States of northern Nigeria the extracts are used to treat burns, skin and wound infections. Further the plant extracts were used to treat various ailments like burns, skin and wound infections, haemorrhoids, constipation, inguinal hernia, intestinal parasitosis, blennorrhagia, syphilis and diabetes⁴.

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MATERIALS AND METHODS

Preparation of the plant material

The healthy leaves of *Cassia alata* were collected from Vedhachalam Nagar, Kanchipuram district, Tamil Nadu, India. The leaves were washed in running tap water and shade dried. The completely dried leaves were ground into fine powder using an electric blender and stored in airtight container till the preparation of various extracts.

Extraction and phytochemical screening

10g of the powdered sample was soaked in 100ml of four different solvents namely, Petroleum ether, Ethanol, Acetone and Chloroform. The flasks were covered with aluminum foil and kept in shaker for 48 h at room temperature. The crude defatted and depigmented extracts were filtered with using muslin cloth and then with Whatman No.1 filter paper. The filtrate was evaporated to dryness and stored in airtight bottles for further use. Preliminary phytochemical screening was done by standard procedure⁵.

Antibacterial susceptibility assays

The test organisms used for the study were four bacterial species which includes; *Bacillus sp.*, *Escherichia coli sp.*, *Staphylococcus sp.*, *Salmonella sp.* The organisms were revived by subculturing them in nutrient broth and incubated for 24 hr at 37°C. An 18 hr culture of the test organism of 0.5 McFarland standards corresponding to approximately 10⁸ cells/ml were used for the assay. Nutrient agar was poured onto a sterile petriplate (90mm diameter) and allowed to stand on a flat bench to solidify. Wells of 6mm diameter and 4mm deep were punched on the agar with a sterile cork borer. Plant extracts were re-dissolved in the corresponding solvents to a concentration of 1mg/ml. The extracts of 10ul volume were poured on the wells and incubated at 37°C for 18hr. The respective solvents were taken as the negative control. Erythromycin (10µg) was used as positive control. The antibacterial activity of the extract was evaluated by measuring the clear zone of inhibition around the wells in millimeter^{6,7}.

Determination of antioxidant activity

DPPH Free radical scavenging assay

The plant extracts were serially diluted from 200ug to 1.075 µg and 0.1mM DPPH solution dissolved in methanol was added. The reactions

were taken in triplicate with DPPH and methanol as control. Ascorbic acid was used as a positive control. The solvent mixtures were covered with a lid and wrapped with aluminum foil and left for incubation in dark for 30 minutes. The absorbance was read in a microplate reader at 515nm. The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula⁸

$$\% \text{ of Inhibition} = ((A \text{ of control} - A \text{ of Test}) / A \text{ of control}) \times 100$$

Where A = absorption at 517 nm

Anticancer activity

The eluted active fractions of *Cassia alata* extracts were studied for their anti proliferative efficacy on the Hep 2 and HCT-15 cells. The sensitivity of the cells was assessed by the MTT colorimetric assay. Percentage of residual cell viability and IC₅₀ values were determined as described earlier⁹ IC₅₀ [1-(OD of treated cells/ OD of control cells)] x 100.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the acetone, chloroform, ethanol, petroleum ether and aqueous extracts of *Cassia alata* leaf were tested to identify the phytoconstituents inherently present in the plant. The extraction resulted in enormous phytochemicals in different solvent systems (Table 1). Petroleum ether could extract alkaloids, steroids, phenol, glycosides and proteins while chloroform extract had terpenoids, flavanoids, alkaloids, steroids and proteins. Acetone extract contained most of the phytochemicals like phenols, flavanoids, steroids, glycosides, saponins, and proteins. Ethanolic extract had the presence of alkaloids, terpenoids, flavanoids, steroids and phenols while aqueous extract had carbohydrates, saponins, phenols and flavanoids.

Antibacterial susceptibility assays – well diffusion

The assay revealed that chloroform extract had a good activity followed by the ethanol and acetone extracts. It was also observed that the chloroform extract had the highest activity against *Bacillus sp.* of 20 mm (Table 2). *Salmonella sp.* is more vulnerable to the chloroform and ethanol extract. All the extracts were able to inhibit the

growth of *Bacillus Sp.* however petroleum ether extract had the least activity against all the organisms.

Antioxidant activity

The free radical scavenging ability of the chloroform, acetone, ethanol and petroleum ether extract of *Cassia alata* was evaluated by DPPH method. The dose response curve represented in Fig 5: shows the radical scavenging effect increases with increase in concentration. The

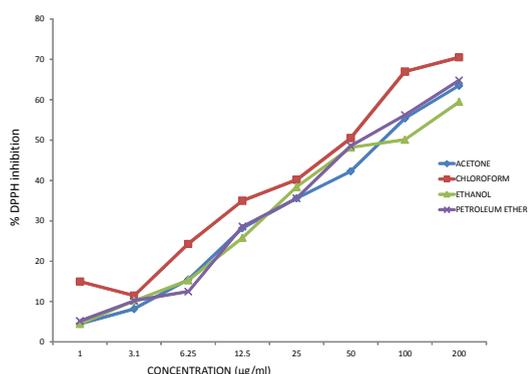


Fig. 1. DPPH free radical scavenging assay of *Cassia alata*

scavenging ability of the plant extracts was evaluated based on their ability to inhibit 50 % (IC_{50}) of the free radicals of the given concentration of DPPH. Table -3 depicts acetone extract has the highest percentage of scavenging ability compared to all the other. The order of reducing ability is acetone >petroleum ether>ethanol>chloroform.

Anticancer activity

The anticancer activity of the crude plant extract was studied against Hep2 and HCT-15 cell

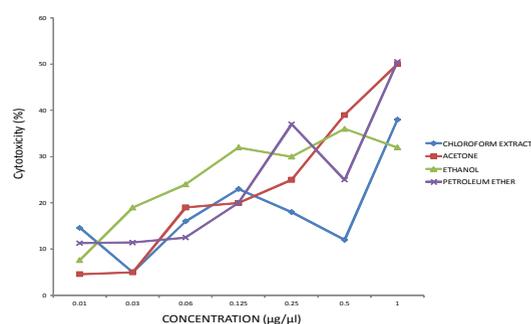


Fig. 2. Effect of *Cassia alata* leaf extracts on Hep2 cell line

Table 1. Phytochemical screening of *Cassia alata* leaf extracts.

S.N	Component	Acetone Extract	Chloroform Extract	Ethanol Extract	Petroleum Ether Extract	Aqueous Extract
1.	Carbohydrates	-	+	-	-	+
2.	Protein	+	+	-	+	-
3.	Phenols	+	-	+	+	+
4.	Saponin	+	+	-	-	+
5.	Glycosides	+	-	-	+	-
6.	Steroids	+	+	+	+	-
7.	Flavonoids	+	+	+	-	+
8.	Terpenoids	-	+	+	-	-
9.	Alkaloids	-	+	+	+	-

(+) indicates presence, (-) indicates absence

Table 2. Antibacterial susceptibility assays – well diffusion assay

S.No	Test Organisms	Petroleum Ether Extract (mm)	Chloroform Extract (mm)	Ethanol Extract (mm)	Acetone Extract (mm)	Antibiotic Disc (mm) [Erythromycin]
1	<i>Bacillus Sp.</i>	8	20	12	15	8
2	<i>E. Coli Sp.</i>	8	8	8	10	20
3	<i>Staphylococcus Sp.</i>	8	10	8	6	8
4	<i>Salmonella Sp.</i>	8	15	12	8	8

line (Fig 2,3). It was observed that the percentage cytotoxicity of the extracts increases with increase in concentration for the HCT-15 cell lines whereas the results were fluctuating for acetone, petroleum ether and chloroform extracts in Hep2 cell line. The IC_{50} value was least for the petroleum ether extract with $0.260\mu\text{g}/\mu\text{l}$ against the Hep2 cell lines.

DISCUSSION

Cassia alata an indigenous plant of biomedical importance was studied with a clear objective of affirming the ethnically claimed activity and to support with the scientific relevance of afore mentioned assignment. Since the choice of the solvent decides the solubility of molecules this experimental design was concerned with a broad spectrum of polarity ranging from non-polar solvent petroleum ether, medium polar solvent chloroform, polar aprotic solvent acetone and polar protic solvent ethanol and water. The ability of the plant to inhibit the growth of microorganisms was tested for both gram positive and gram negative organisms. To sum up the chloroform extract had a good antibacterial activity followed by the ethanol and acetone extracts. We also observed that the plant

extracts had good antibacterial activity against the gram positive bacteria than the gram negative ones. The presence of flavonoids, terpenoids and alkaloids may be an attributing factor for the better activity of chloroform extract. From this result we could infer that the active molecule could be of polar nature as they were extracted in the solvents of corresponding polarity. Organic solvents are the best suitable medium for the extraction of active phytoconstituents which is in unison with the results of Ehiowemwenguan *et al*, 2014¹⁰. The rich presence of phytochemicals made to study antioxidant activity. The study demonstrated that the acetone extract had comparatively better performance and it was reasonable to predict their activity as they had the presence of phenolic compounds and flavonoids whose biological activity is of broad spectrum. It is understood these compounds distinctly scavenged the free radicals with protons donated from the hydroxyl groups of the phenolic molecules¹¹. Flavonoids are reported to have a significant role on membrane permeability and inhibition of enzymes like ATPase and phospholipase A_2 which may also be a contributing factor for the observed antioxidant activity¹². Oxidative free radicals generated during the metabolic process are one of the proven causatives of diseases like cancer, arthritis, asthma and many more. The pronounced antioxidant ability of the plant provoked the assumption that the plant also could have a role in cancer prevention and the study was carried out using Hep2 and HCT-15 cell line. The anticancer activity increases with increase in concentration on the HCT-15 colon cancer cell lines for the acetone, petroleum ether, chloroform and

Table 3. Scavenging activity of the plant extracts

S N	Plant Extract	IC_{50} Value
1	Acetone	82
2	Chloroform	51
3	Ethanol	72
4	Petroleum ether	75

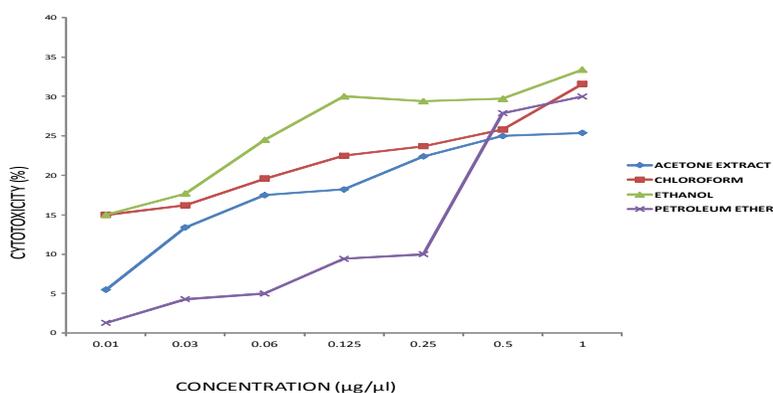


Fig. 3. Effect of *Cassia alata* leaf extracts on colon cell line

ethanol extracts. The plant extract as such are a crude composite of diversified chemical which inhibit the proliferating cells in multiple mode either as agonist, antagonist or synergist. The continuous raise in the chemotherapeutic drug resistance have provoked the use of mixture of complex molecules in the treatment which could target multiple targets¹³. The cytotoxicity activity for the ethanol, petroleum ether, chloroform extracts showed a nonlinear response on Hep2 cells. The lower activity for the three extracts may be attributed to the lower affinity to the molecular targets or inability of the molecule to permeate inside the proliferating cells. Similar reports of plants being an indigenous source of anticancer molecules are reported for the methanolic extract of roots of *Glochidion zeylanicum* on HepG2, HT-29 and PC-3¹⁴. Also the methanolic extract of leaves of *Argemone mexicana* was found to be active against HeLa and MCF-7 cells¹⁵. Species of *Ononis hirta*, *Inula viscosa*, *Salvia pinardi*, *Verbascum sinaiticum* and *Ononis sicula* were also studied for their anticancer activity on Hep-2, MCF-7, and Vero cell lines¹⁶. Although the success rate of discovering a new molecule for cancer treatment is a big challenge owing to the tumour heterogeneity, plants based phytochemicals have their great contribution as antineoplastic candidates and providing a potential source for the drug industry¹⁷. Further in vivo studies with experimental analysis would reveal the phytochemical responsible for the bioactivity of the plant and characterization of it would recommend the plant to be used by the drug industry.

CONCLUSION

The plant *Cassia alata* taken for our study is found to have antibacterial and anti-cancer activities. The bioactivity of the plant should be further investigated with active fractions purified from the crude extracts and the new investigatory molecule should be characterised for its structure and its relative activity which comprehend the invitro activity studies.

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