

EVALUATION OF *IN VITRO* ANTICANCER POTENTIAL OF ETHANOLIC EXTRACT AND ITS DIFFERENT FRACTIONS OF *CAESALPINIA BONDUC* (L) ROXB SEEDS

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ABSTRACT

Objective: The present study aims to evaluate the anticancer potential of ethanolic extract and its different fractions of *Caesalpinia bonduc* seeds against Ehrlich Ascites Carcinoma (EAC) cell lines.

Methods: Ethanolic extract and its fractions were subjected to preliminary phytochemical screening and anticancer activity by using *in vitro* anticancer assay. Chemical constituents of petroleum ether fraction of *C. bonduc* seeds were identified by using GC-MS and the active compounds were subjected to *in silico* studies.

Results: *In vitro* anticancer assay showed that the petroleum ether fraction of ethanolic extract of *Caesalpinia bonduc* seeds has potential anticancer activity.

Conclusions: Petroleum ether fraction of ethanolic extract of *Caesalpinia bonduc* seeds has significant anticancer activity. Further in-depth studies, could result in the development of a good anticancer agent from the seeds of *Caesalpinia bonduc*.

Keywords: *Caesalpinia bonduc*, Anticancer, *In vitro*, *In silico*.

INTRODUCTION

Plants play a vital role in maintaining human health and quality of life. Herbal treatment is based on presence of phytoconstituents which are responsible for treatment of diseases [1]. The focus on medicinal plant research has been increased worldwide because of the belief that "green medicine" is safe and cheaper than synthetic drugs [2].

Cancer is one of the most serious threats causing death in India, United States and Western world. There is an anomalous number of plant derived anticancer drugs with diverse structural types are used in both clinical use and also undergoing clinical trials. This suggests that plants will continue to be an imperative and prized resource for anticancer drug discovery.

The World Health Organization (WHO) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been in existence, often for thousands of years [3]. Herbal plants play a significant role not only in diseased conditions but also in maintaining human health and improving the quality of human life for thousands of years.

More than 3000 plant species are reported to be used for cancer treatment. In recent years, secondary metabolites of plants (phytochemicals) have been extensively investigated as a source of medicinal agents. They have been used as a homeopathic remedy and have been reported for its successful treatment of certain type of cancers. Plant extracts have the ability of activating the apoptotic pathway of cancer cells and doesn't have any ethical issues when it is used as drug formulations as it is purely herbal.

Caesalpinia bonduc is a medicinal plant belonging to the family Caesalpiniaceae. It is popularly used in the traditional Indian system of medicine. It is found in India and tropical countries of the world. It is reported to have therapeutic properties like anti-asthmatic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-bacterial, anti-filarial, anti-tumor and anxiolytic activity [4 & 5]. The plant *Caesalpinia bonduc* is used to treat rheumatism, pyretic, inflammatory, helminthiasis, infectious diseases, estrogenic disorders, diabetes, fever [6], malaria, skin diseases, leprosy, paralysis and neuro disorders [7].

MATERIALS AND METHODS

Plant material

The seeds of *Caesalpinia bonduc* (L.) Roxb. were procured from the market, Thanjavur and the seed materials were identified and authenticated by Dr. P. Brindha, Carism, SASTRA University, Thanjavur.

Preparation of plant material

Caesalpinia bonduc (L.) Roxb. seeds were cleaned, dried and then mechanically powdered.

Extraction

1000g of seed powder was soaked in 98% ethanol for 48 hours. The ethanolic extract of *Caesalpinia bonduc* was filtered using whatmann filter paper No.1. The extract was concentrated using vacuum evaporator. The ethanol extract was partitioned by using petroleum ether fraction, chloroform, ethyl acetate and ethanol successively.

Preliminary phytochemical analysis

Preliminary phytochemical screening was performed to identify the phytoconstituents. Ethanolic extract and its fractions revealed the presence of biologically active compounds such as phenolics, flavonoids, quinines, saponins, alkaloids, glycosides and tannins [8].

In vitro anticancer studies

Maintenance of cells

Ehrlich Ascites Carcinoma (EAC) cell lines were maintained in Central Animal Facility, SASTRA University and were maintained by weekly intraperitoneal inoculation of 1×10^6 cells/mouse [9].

Mtt assay

EAC cell lines were cultured in 96 well plates with growth medium RPMI1640 and 10% FCS. Dissolved 1mg of sample (ethanolic extract, ethyl acetate fraction and ethanol fraction) in 100 μ l of water and 1mg of sample (petroleum ether, chloroform fractions) in 1% DMSO. Sample was filtered through 0.45 μ m syringe filter. 10,000cells/100 μ l media were incubated with increasing

concentrations of drug for 48 hrs at 37 °C in CO₂ incubator with 5% CO₂. After incubation, 20 µl of MTT (5mg /ml conc.) was added and then incubated for 3 hrs at 37°C in CO₂ incubator. The Purple colour precipitate was formed. 10 µl of DMSO was added in all the wells to dissolve MTT formazon crystals and incubated for 10 min at 37°C in CO₂ incubator. The absorbance was recorded at 590 nm [10].

$$\% \text{ Anticancer activity} = \frac{100 - \text{Treated sample OD} \times 100}{\text{Control OD}}$$

Gc-ms analysis

Perkin Elmer Clarus 500 GC-MS instrument was used for the analysis of the compounds present in fraction under study. Capillary column made of Elite-5ms (5% phenyl 95% dimethyl polysiloxane) was used. The oven program was fixed to 50°C @ 7°C/min to 220°C (2 min), @ 7° C/min to 280°C (10 min) and injector temperature was 280°C.

Helium was used as carrier gas at the flow rate of 1 ml/min. Sample was injected and the compounds obtained were matched using the library NIST 2005.

In silico analysis

To support the anticancer activity, the *in silico* approaches has been implemented in which the docking software autodock was used. The target molecule was chosen to be Bcl-2 as it is a major gene that codes for a large family of apoptosis regulating proteins. Compounds obtained from GCMS were docked with Bcl-2 and the results were found to be significant.

RESULTS

The amount of ethanol extract obtained from seed powder (1000g) of *Caesalpinia bonduc* is 37.029g. The ethanol extract was partitioned by using increasing polarity of solvents such as petroleum ether fraction (8.577g), chloroform fraction (8.676g), ethyl acetate fraction (0.6g) and ethanol fraction (9.811g) successively. Preliminary phytochemical analysis of ethanol extract and the four fractions of *Caesalpinia bonduc* seeds were shown in table 1.

The % anticancer activity was evaluated for ethanolic seed extract and four fractions, which showed the anticancer property of *Caesalpinia bonduc* (Table 2 and Fig 1).

Table 1: Preliminary phytochemical analysis of ethanol extract and the four fractions of seeds of *Caesalpinia bonduc*

Phytoconstituent	Ethanol Extract	Pet-ether Fraction	Chloroform Fraction	Ethyl acetate Fraction	Ethanol Fraction
Phenolics	-	-	-	-	+
Flavones	+	-	+	-	+
Glycosides	+	-	+	+	-
Saponins	-	+	-	-	-
Steroids	-	-	-	-	-
Alkaloids	+	+	+	+	-
Anthraquinones	-	-	-	-	-
Quinones	+	-	+	-	+
Tannins	+	-	+	-	-

In vitro anticancer studies (MTT Assay)

Table 2: % Anticancer activity of ethanolic seed extract and four fractions of *Caesalpinia bonduc*

Concentration (µg/ml)	% Anticancer activity				
	Ethanol Extract	Pet ether Fraction	Chloroform Fraction	Ethyl acetate Fraction	Ethanol Fraction
1000	66.19±0.37	66.96±0.59	67.50±0.16	67.61±1.73	51.84±0.97
500	53.03±1.02	55.71±1.24	55.29± 0.97	55.02±0.70	39.59±0.64
250	50.92±2.49	52.53± 4.22	54.48± 1.67	51.30±4.54	34.00±0.75
125	40.05±0.54	49.20±0.16	47.82±7.84	45.87±1.19	32.74±2.21
62.5	37.22±1.62	46.67±2.76	38.40±0.92	44.57±1.08	31.67±3.41
31.25	30.82±1.24	45.33±0.97	34.31±2.70	44.22±0.92	28.15±2.32
15.625	29.14±3.19	43.88± 0.21	29.75±2.32	39.09±3.62	19.69±0.00
7.8125	17.31±4.33	38.14± 3.68	23.55±0.16	36.64±3.51	16.81±1.78

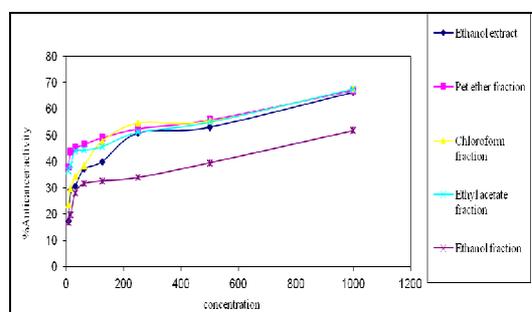


Fig. 1: Graphical representation of MTT assay between Concentrations of the drug vs %anticancer activity.

IC₅₀

IC₅₀ values were obtained by plotting graph between concentrations of drug vs % Anticancer activity that indicated in

table 3. Pet-ether fraction has shown the least IC₅₀ value, which indicates that pet -ether fraction has highest anticancer activity compared to other samples.

Table 3: IC₅₀ values for ethanol extract and its four fractions of *Caesalpinia bonduc* seeds

Sample	IC ₅₀ µg/ml
Pet- ether Fraction	120
Chloroform Fraction	140
Ethyl acetate Fraction	190
Ethanol Fraction	220
Ethanol Extract	900

Gc-ms results

From IC 50 values, petroleum ether fraction was found to have higher anticancer property and hence it was subjected to GCMS, in order to understand the compounds present in the fraction. GC-MS

profile of Petroleum ether fraction of *Caesalpinia bonduc* seeds was shown in Fig 2.

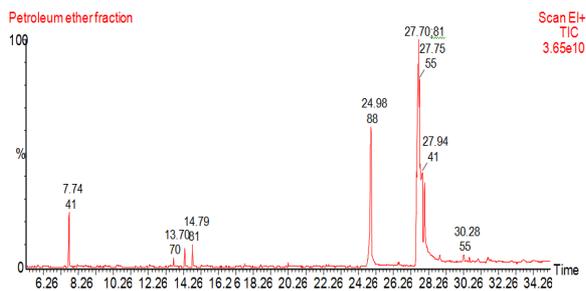


Fig 2: GC-MS profile of Petroleum ether fraction of *Caesalpinia bonduc* seeds chromatogram (x-axis = Retention time, y-axis = % intensity/%abundance)

In silico analysis

The cyclic compounds obtained from the GCMS analysis of petroleum ether fraction of seed extract of *caesalpinia bonduc* were n-Butyric acid tetrahydrofurfuryl ester, Cis11-Hexadecenal, 1H-Naphtho[2,1-b] pyran,3-ethenyl dodecahydro-3,4a,7,7,10a-penta methyl. In order to understand the anticancer potential of the compounds present in the fraction, these compounds were docked with Bcl-2 anti-apoptosis protein which showed the binding energy of each of the compounds. It was understood that 1H-Naphtho[2,1-b]pyran,3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl with least binding energy (-6.42 kcal/mol) was considered the best protein-ligand complex fit. This was because of the lowest amount of energy needed by 1H-Naphtho[2,1-b]pyran,3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3à,4aà,6aà,10aà,10bà)]- to bind with the Bcl-2 protein receptor and cause anti-apoptosis thereby successfully killing EAC cell lines. The energies of these compounds with Bcl-2 protein were tabulated in table 4. Pictorial representations of docking of compounds with Bcl2 were shown in Fig 3, 4 and 5.

Table 4: Docking of compounds in the pet ether fraction of seed extract of *Caesalpinia bonduc* with Bcl2

Ligand	Binding Energy(kcal/mol)
n-Butyric acid tetrahydrofurfuryl ester	-3.88
Cis11-Hexadecenal	-1.86
1H-Naphtho[2,1-b]pyran,3ethenyldodecahydro-3,4a,7,7,10a-pentamethyl,3R-(3à,4aà,6aà,10aà,10bà)]-	-6.42

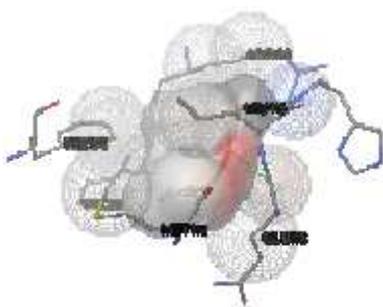


Fig. 3: Pictorial representation of docking of n-Butyric acid tetrahydrofurfuryl Hexadecenal with Bcl-2.

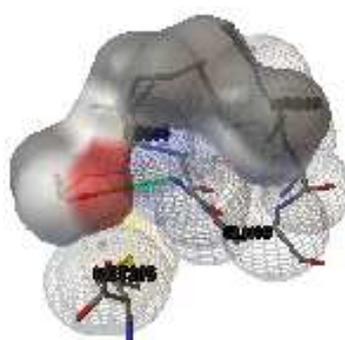


Fig. 4: Pictorial representation docking of Cis11- Hexadecenal with Bcl-2.



Fig. 5: Pictorial representation of 1H-Naphtho [2,1-b]pyran,3-ethenyl dodecahydro-3,4a,7,7,10a-pentamethyl-, [3R-(3à,4aà,6aà,10aà,10bà)] with Bcl-2.

DISCUSSION

The test for *in vitro* anticancer (MTT assay) revealed the antitumor potential of seed extract of *Caesalpinia bonduc*. The % anticancer activity was increasing with increase in concentration of seed extract. The petroleum ether fraction, the concentration was ranging from 7.8125 to 1000µg/ml shows the significant % of cytotoxicity was obtained were 38.1412 % and 66.96%. This indicates the higher therapeutic values of the Petroleum ether fraction showed the least IC₅₀ value compared to other samples.

The phytochemical analysis of ethanolic extract and the fractions of *Caesalpinia bonduc* seeds showed the presence of phenols, flavones, alkaloids, glycosides, quinones, saponins and tannins. The pet ether fraction of *Caesalpinia bonduc*, when subjected to GCMS analysis (Fig 2) showed the presence of various phytochemical compounds from which 3 cyclic compounds were identified. They were docked into the anti-apoptotic protein Bcl-2. Docking shows the binding energy of each of the compounds with Bcl-2 protein. It was understood that 1H-Naphtho[2,1-b]pyran,3-ethenyl dodecahydro-3,4a,7,7,10a-penta methyl with least binding energy (-6.42 kcal/mol) was considered the best protein-ligand complex fit. This was because of the lowest amount of energy needed by 1H-Naphtho[2,1-b]pyran,3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl to bind with the Bcl-2 protein receptor and cause anti-apoptosis thereby successfully killing EAC cell lines. Hence from the current study it is evident, that

the pet ether fraction of *Caesalpinia bonduc* seeds can be developed as a potent anticancer drug.

CONCLUSION

In vitro anticancer assay showed that the petroleum ether fraction of ethanolic extract of *Caesalpinia bonduc* seeds has potential anticancer activity. It has shown high potency in killing EAC cell lines through induced apoptosis. Hence, this work could be the silver lining in further researches in anticancer herbal therapy against other breast cancer cell lines.

Further, *in silico* approaches also indicate that the compound 1H-Naphtho[2,1-b]pyran,3-ethenyldodecahydro-3,4a,7,7,10a-pentamethyl from pet ether fraction showed the least binding energy for protein-ligand interaction, which can be obtained and purified in the future as a potent drug against EAC cell lines.

This work has confirmed that herbal remedies are effective in treating cancer. It was concluded that seed extract of *Caesalpinia bonduc* was effective against EAC cell lines and can be developed as a drug for treating Ehrlich ascites carcinoma cell lines in the near future.

CONFLICT OF INTERESTS

Declared None

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