STUDY ON THE PHYTOCHEMICAL PROPERTIES OF SIMAROUBA GLAUCA AND ISOLATION OF FLAVONOID AND ITS EFFECT ON ANTICANCEROUS PROPERTIES

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Abstract—*Medicinal plants also called herbs, botanical drugs or natural product drugs have been discovered and used in traditional medicine practices since pre-historic times. Simarouba glauca commonly known as Lakshmi Taru towards the southern part of India which belongs to the family Simabaracia is a medicinal plant. A phytochemical study on the liquid leaf extract is carried out and various pharmacologically important components such as flavonoids, phenol, saponin, etc are identified. However, a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine is uncertain. Flavonoids are a group of poly-phenolic compounds that occur naturally in foods of plant origin and are categorized, according to its chemical structure. The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. In this paper antioxidant, antitumor and antimicrobial properties of flavonoid isolated from Simarauba glauca are studied.*

Keywords: Antioxidant properties, Antimicrobial properties, antitumor properties, Flavonoid, Phytochemical study, *Simarauba glauca*

1. INTRODUCTION

Varieties of plants are used as a 'source of medicine' since ancient practice, as it is an important component in the health system. Medicinal plants are identified on the basis of their biological parameters such as taste, metabolic property, quality, etc. *Simarauba glauca* commonly called Lekshmi taru belongs to the family *Simaraubacea*, which is a medium sized plant that spreads around 25-30 feet. Extract of *Simarauba glauca* has a wide range of medicinal properties hence could be used for treating several diseases.

In this research work the methanolic and water extracts of the leaves of *Simarouba glauca* were prepared with the help of simple extraction and soxhlet extraction. This extracts were used to detect the presence of different phytochemicals like alkaloids, phenol, flavanoid, tannin, etc. These flavanoids which are naturally occurring polyphenolic metabolites regarded as safe and easily obtainable, that makes them ideal candidate for chemo prevention and associated agent in chemical treatment. Chemo reagents that are widely used for clinical treatment are highly toxic to produce severe damage to normal cells. An ideal anticancer agent is the one that exerts minimum toxicity to kill tumor cells. Due to polyphenolic structure, flavonoids possess anti and pro oxidant activity. Cancer cells exhibit a higher and more peristant oxidative stress level compared to normal cells, rendering malignant cells more vulrunable to being killed by drugs and certain phytochemicals such as flavonoids.[1]

2. EXPERIMENTAL SETUP

2.1 Collection of plant materials

Fresh leaves of *Simarauba glauca* were collected from the campus of Sree Buddha College of Engineering and the leaves are washed using distilled water a part of it was kept in shade for drying for around a week. The fresh leaves are used for water extraction whereas the dried powder is prefer for alcoholic extraction.

Fig 1. Leafs of Simarauba glauca collected from the campus

2.2 Preparation of water extract

10 g fresh leaves were cut and boiled in 100ml of distilled water for around 20 minutes at 60^0 C. A green coloured solution of extract was then obtained by filtration using Whatmann filter paper No.1

Fig II. Water extract

2.3 Preparation of alcoholic extract

Dried leaves were powdered using grinder and the powdered leaves were loaded in soxhlet extraction apparatus. The extraction was done with ethanol. 10 gram of plant material was subjected for 40 ml Ethanol at 65 ^{0} C temperature and extraction was carried out for 5 hours. The colour of the extract was dark green. The obtained liquid extracts were subjected to Rotary evaporator and subsequently concentrated under reduced pressure (in vaccum at 40°C) and evaporated to dryness and stored at 4°C in air tight bottle.[1]

2.4 Preliminary qualitative phytochemical screening:

Initially, the extracts were subjected to qualitative and quantitative analysis for various phytochemical constituents including alkaloids, proteins, phenols, tannins, flavonoids, glycosides and saponins. [2]

2.4.1 Test for alkaloid:

Equal volumes of solvent extract and Wagner's reagent were placed in a test tube and incubated for some minutes. The presence of alkaloid was indicated by a brown precipitate

2.4.2 Test for Phenol:

2ml of extract was added to 2ml of ferric chloride solution, a deep bluish green solution will be formed indicating presence of phenols

2.4.3 Test for flavonoid:

5cm^3 of the solvent extracts was placed in a test tube and few pieces of magnesium chips were added, followed by concentrated hydrochloric acid in drops And then in excess. Formation of reddish colour indicates the presence of flavonoids.

2.4.4 Test for glycoside:

25 ml of dilute sulphuric acid was added to 5 ml of the extract in a test tube and boiled for 15 minutes, cooled and neutralized with 10% NaOH, and then 5 ml of Fehling solution A and B was added. A brick red precipitate of reducing sugar indicates the presence of glycosides.

2.4.5 Test for saponin:

1g of the sample was weighed into a conical flask in 10ml of sterile distilled water was added and boiled for 5 min. The mixture was filtered and 2.5ml of the filtrate was added to 10ml of sterile distilled water in a test tube. The test tube was stopped and shaken vigorously for about 30 second. It was then allowed to stand for half an hour. Formation of honeycomb froth indicates the presence of saponins.

2.4.6 Test for tannin:

3g of the powdered sample was boiled in 50ml distilled water for 3minutes on a hot plate. The mixture was filtered and a portion of the filtrate diluted with sterile distilled water in a ratio of 1:4 and 3 drop of 10% ferric chloride solution added. A blue or green colour indicates the presence of tannins.

2.4.7 Test for protein:

1ml of extract was shaken with about 2ml of million's reagent. A brick-red colouration indicates the presence of protein.

3. Extraction and Isolation of flavonoid

Dried sample were extracted using soxhlet extraction in 80% methanol (100ml/g dry weight) for 24hrs. Ethyl acetate was used as the extraction medium. Column chromatography was carried out to elute out the crude sample using ethyl acetate as it is supposed to contain highest amount of flavonoid. The fractions are collected and then dried.[4,5]

3.1 Confirmation test of flavonoid

95% ethanol and few drops of concentrated HCl are added to the extract. 0.5g of Magnesium chips were also added to the solution. Observation of pink colour indicates presence of flavonoid. [4,6]

3.2 Identification of flavonoid by TLC

TLC was performed to identify flavonoids. The extract was spotted on the lower side of the TLC plate (20x20 cm) coated with silica gel. The TLC was allowed to run one dimensionally in the mobile phase solvent (ethyl acetate-methanol-water, 5:1:5) at room temperature. The paltes are then visualized under UV light.[7]

3.3 Free radical DPPH Radical scavenging Assay

The ability of flavonoids isolated from Simarauba glauca leaves to scavenge the stable DPPH radicals are estimated by using Mensor method, where 0.1mM solution of DPPH is prepared using methanol solution and extract of leaves. The mixture was shaken continuously and absorbance value was measured at 517nm. A lower absorbance value indicates higher scavenging actrivity. The ability of DPPH scavenging was calculated by using the formula

$$[(A_0 - A_1)/A_0]x100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the extract. [3]

3.4 Cytotoxicity activity by MTT assay

The cytotoxicity assay was carried out using MCF-7 human breast carcinoma cells maintained as monolayer cultures. The extract was diluted in culture medium at different concentrations. [6]

4 Antimicrobial effect of Flavonoid

4.1 Selection of microbial strain

The strains of *Escherichia coli* and *Pseudomonas aeruginosa*were used obtained from the research lab of Sree Buddha College of Engineering and used for identifying the antimicrobial activity of the extract.

4.2 Antibacterial assay

Antimicrobial susceptibility testing was done using the well-diffusion method according to the standard of the National Committee for Clinical Laboratory Standards. The plant extracts were tested on petri plates to detect the presence of antibacterial activity. Prior to streaking a sterile borer is used to punch 5mm diameter wells into the medium. Excess inoculum is removed by dipping a cotton swab into the suspension, rotated several times and pressed firmly on the inside wall. The surface of the agar plate was streaked over the entire sterile agar surface rotating the plate to ensure an even distribution of inoculum with a final swab around the rim. The plates are allowed 3 to 5 min to dry the excess moisture.

Aliquots of each test extract were dispensed into each well after the inoculation of the plates with bacteria. The plates are sealed with parafilm, labeled, and placed in an incubator set to 37°C. After 24 hours of incubation, each plate was examined for inhibition zones. A ruler was used to measure the inhibition zones in millimeters. Every experiment was carried out in parallel, and the results represented the average of at least three independent experiments

5. RESULT ND DISCUSSION

5.1 Phytochemical Analysis

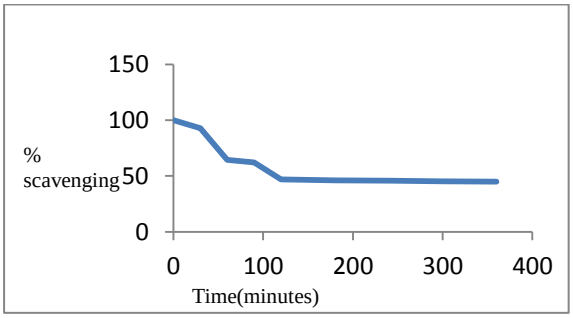
Phytochemicals are secondary metabolites and are found in most of the medicinal plants. They have the ability to produce phytological effect on human body. Leaf extracts isolated by simple water extraction and soxhlet extraction is compared.

TABLE 1.1 PHYTOCHEMICAL ANALYSIS OF WATER AND ETHANOLIC EXTRACT

S/N	Chemical constituent	Method	Ethanolic extract	Water extract
1	Alkaloid	Wangers	✓	✓
2	Phenol	Ferric chloride	✓	✓
3	Flavanoid	Magnesium chips	✓	✓
4	Glycoside	Fehlings	✓	✓
5	Saponin	Emulsifying	-	-
6	Tannin	Ferric chloride	✓	✓
7	Protein	Millons	✓	✓

5.2 Antioxidant activity of leaf extract

DPPH assay is one of the valid and easy way to evaluate scavenging activity of antioxidants, since the radical compound is stable and does not generate other radicals. DPPH radicals react with the reducing agent and electrons become paired and the solution losses color with the gaining of electrons. The decrease in the DPPH concentration radical is due to the scavenging of ethanol extract. The scavenging activity has been reduced to 48%

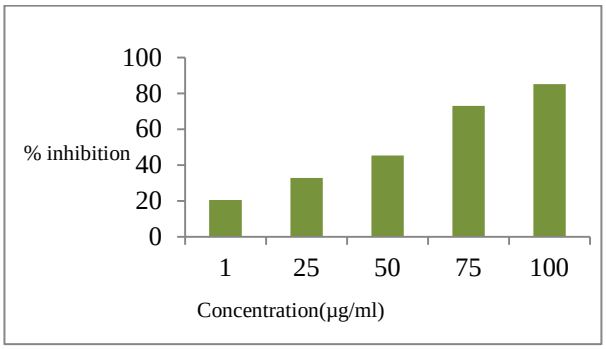


Graph 1.1 DPPH scavenging activity of the leaf extract

5.3 Cytotoxicity against MCF-7 Human breast cancer cell line

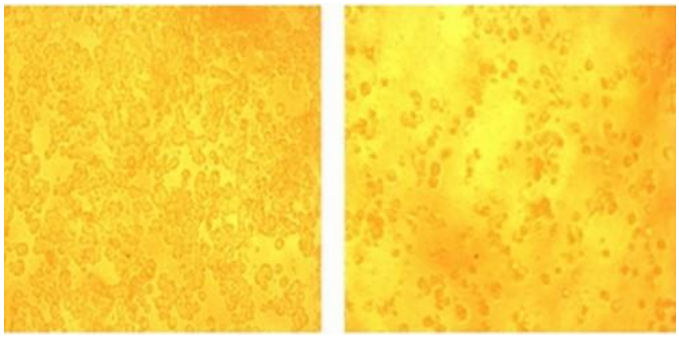
The flavonoid isolated from the leaves of Simarauba glauca was tested for its in vitro cytotoxicity against MCF-7,

Human breast cancer cell line. The inhibitory concentration at 50% growth (IC_{50}) value was found to be 54.5µg/ml



Graph1.2: Cytotoxicity activity of flavonoid isolated from Simarauba glauca against MCF-7 Human Breast Carcinoma cell line

The anticancer effect of flavonoid is due to the ability to induce apoptosis of tumor cells. Most breast cancer cells contain ER-positive and ER-negative cells. Agents that can induce the growth of both cells are of high interest.



Normal MCF-cell line Toxicity-100µl

Fig IIICytotoxicity activity of flavonoid isolated from Simarauba glauca against MCF-7 Human Breast Carcinoma cell line

5.4 Antibacterial assay of flavanoid

TABLE 1.2 INHIBITION ZONE OF ANTIBACTERIAL ASSAY

S/N	Microorganism	Zone of inhibition
1	Pseudomonas	14.0 mm
2	Ecoli	3.0 mm

6. CONCLUSION

In conclusion this present study shows that the tree contains various phytochemicals and metabolites which has a role in the insecticidal, anti-bacterial properties. It could be used for the production of different plant based medicines. But also there is ample need to work on to improve the quality and quantity of the products. Bioactive flavonoid is isolated from Simarabau glauca and was confirmed by chromatography and qualitative techniques. Isolated flavonoid showed potential anticancer activity. Further work is certainly needed to develop and produce novel drugs from natural sources introducing structural variations into the backbone of flavonoids and modifying their structures to further improve biological activity and exhibit more potent anticancer effects.

7. ACKNOWLEDGEMENT

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8. REFERENCES

[1] Ashwanikumar, VishwaRawat, Amardeep and Vikaskumar, Comparative evaluation of phytochemicals in Methanolic and Ethanolic leaf extracts of Anticancer paradise Tree Simarauba glauca, volume5(2016) pp. 679-686

[2]. KumariSmita, S.K. Sarangi, K. Manjunath, Phytochemical analysis of leaf extracts of antihyperglycemic medicinal plants, Int J Pharma Bio Sci 2017 October, 8(4), pp 212-218

[3]. Vijayalakshmi A, Masilmani K, Nagarajan E andRavichandiran V, In vitro antioxidant and anticancer activity of flavonoids from Cassia Toralinn leaves against human breast carcinoma cell lines, Der pharma chemical, 2015,7(9), 122-129

[4]. Jobyjose, SudheeshSudhakaran, Sumesh Kumar T.M, Sony Jayaraman, E. JayadeviVariyar, A comparative evaluation of anticancer activities of flavonoids isolated from Mimosa Pudica, Aloe vera and PhyllanthusNiruri against Human breast carcinoma cell line (MCF-7) using MTT assay, volume 6, issue 2,2014, 319-322

[5]. Mahesh Chand Meena, VidyaPatni, Isolation and Identification of flavonoid from CitrulluscolocynthisSchard. Asian J. Exp.Sci.2008, 22(1), 137-142

[6]. Mahesh AR, Ranganath MK, Harish kumar DR, Enrichment of flavonoids from the methanolic extract of BoerhaaviaDiffusa Roots by partioning Technique. Res.J.Chem.Sci, 2013, 3(1), 43-47

[7]. Sathish Kumar T, Sampath M, Sivachandran S.V, Shanmugam S, Rajasekaran P, Optimal process for the extraction and identification of flavanoids from the leaves of PolyalthiaLongifolia using L16 orthogonal design of experiment, International Journal of Biological and Chemical Sciences 2009, 3, 736-745