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PHYTOCHEMICAL ANALYSIS AND *INVITRO* ANTICANCER ACTIVITY OF ETHANOL EXTRACT OF *WRIGHTIA TINCTORIA*

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Abstract*: The present study examines the nature of phytoconstituents and in vitro anticancer activity of ethanol extract of Wrightia Tinctoria leaves. The preliminary phytochemical screening conducted by usual chemical tests indicated the presence steroids, alkaloids, terpenoids, flavonoids, glycosides and phenolic compounds. GC-MS analysis of Wrightia Tinctoria ethanolic extract revealed the presence of eighteen compounds. In the present study, the major chemical constituents are 2,5-Dimethoxybenzaldehyde oxime (9.52%), 5,5''-Dibromo-4'-methoxymethoxymethyl-2,2',6',2''-terpyridine (6.31%), 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole (4.68%) and 2-Ethyl-5-propylpyridine (4.23%). The in vitro anticancer activity was tested against lung cancer (NH40) cell line using MTT assay. The ethanol extract of W. Tinctoria showed dose dependent anticancer activity. The IC_{50} value was 100µg/ml. The result revealed that W. Tinctoria plant has significant anticancer activity.*

Keywords*: Wrightia Tinctoria, GC-MS, in vitro anticancer activity, NH40 cell line, MTT assay*

I. Introduction

Wrightia Tinctoria belongs to the family Apocynaceae is a small deciduous tree with pale grey, smooth bark, distributed in tropical Africa and Asia. The plant is commonly known as Paalai and generally called "Sweet Indrajao".[1] The juice of the tender leaves is used an effective drug for treatment of jaundice.[2] The crushed fresh leaves when filled in the cavity of decayed tooth relieve toothache. The leaves are a fodder for the cattle, goat and sheep. In south India the plant is used for green manuring rice fields.[3] The leaves of this tree yield a blue dye called pala indigo.[4] *W. Tinctoria* leaves were soaked in coconut oil for few hours and applied for eczema psoriasis and other skin diseases.[5] The *W. tinctoria* flower has been reported to have a good anti-inflammatory activity.[6] Bark and seeds are used to cure bilious infections, psoriasis, leprosy, asthma and various skin diseases.[7] Many authors reported pharmacological activity of *W. Tinctoria* plant like antiulcer activity, anti-inflammatory activity, wound healing activity, anthelmintic activity and antimicrobial activity.[8-11]

There is limited work on anticancer activity. So our main objective of the present work is to evaluate the preliminary phytochemical screening and the anticancer activity against NH40 cancer cell line using MTT Assay.

II. Materials and Methods

Plant Material

Fresh leaves of *W. tinctoria* were collected from area near Udumalpet, Tamilnadu, South India between the periods of September to October. The plant sample was authenticated by the Botanical Survey of India, Coimbatore, Tamil Nadu.

Extraction process

The collected leaves (500 kg) were dried in shade for 10 days. Air dried leaves of *W. tinctoria* was chopped into small pieces. The crude powered material was defatted with ethanol by cold macerated for three days with occasional shaking. The extract was then subjected to vacuum distillation and was concentrated to yield a greenish residue (5 g).

Preliminary Phytochemical Screening

The ethanol extracts of leaves of *W. tinctoria* were subjected to qualitative chemical analysis to identify the nature of phytochemical constituents present.[12]

GC-MS Analysis

GC-MS analysis of the ethanol extract of *W. tinctoria* was carried out in thermo GC-trace ultra version: 5.0 coupled with thermo MS DSQ II instrument. The compounds were separated on DB-35, MS capillary DSQ II instrument. Compounds were separated on DB-35, MS capillary standard non-polar column (0.25mm), film thickness 0.25µm. Helium was used as the carrier gas and the temperature programming was set with initial oven temperature at 70ºC and held for 2 minutes and the temperature of the oven was raised to 260ºC for 10 minutes, raised 6ºC per minute and the final temperature was 300ºC for 10 minutes.

Identification of phytoconstituents

The components were identified by comparison of their mass spectra with those of National Institute of Science and technology (NIST) mass spectral library version 2.0d, as well as on their comparison of their retention time either with those of authentic compounds or with their literature values.

***In vitro* anticancer activity by MTT assay**

The *in vitro* anticancer activity of ethanol extract of *W. tinctoria* leaves were tested against NH40 lung cancer cell line using MTT assay. The ethanol extract was diluted to different concentrations via 5µl, 25µl, 50µl, 75µl, 100µl. The cell viability of cancer cells after inhibition was noted.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37ºC for 4 hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570nm using micro plate reader. The % cell inhibition was determined using the formula

$$\% \text{ cell inhibition} = 100 - [\text{Abs (sample)}/\text{Abs (control)}] * 100$$

IC_{50}value was determined by Nonlinear regression graph was plotted between % cell inhibition and $\log_{10}$ concentration.

III. Results and Discussion

Preliminary phytochemical screening

From the chemical tests, the nature of phytochemical constituents present in the ethanolic extract of *Wrightia tinctoria* was given in the table 1. The result agrees well with the literature report.[12]

Table 1: Preliminary phytochemical screening of ethanolic extract of *W. tinctoria* leaves

S.No	Phytoconstituents	phytoconstituent
1.	Alkaloids	++
2.	Phenolic Compound	++
3.	Flavanoids	++
4.	Tannins	++
5.	Terpenoids	++
6.	Steroids	++
7.	Glycosides	++

GC-MS Analysis

The GC – MS is used to identify the compounds like Terpenes, hydrocarbons, fatty acids and branched chain hydrocarbons. The GC-MS analysis of the ethanol extract of *W. tinctoria* leaves showed the presence of 18 chemical constituents. The compounds were characterized and identified by comparing the GC-MS spectra of the constituents with the NIST library along with the parameters which includes the retention time, peak area, molecular weight. Among the 18 compounds identified the most popular compound is 2, 5-Dimethoxybenzaldehyde oxime (37.52) and the minor compounds include 5,5”-Dibromo-4’-methoxymethoxymethyl-2,2’:6’,2”-terpyridine (34.28), 3-Dodecen-1-al (20.42), 4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole (31.53), 2-Ethyl-5-propylpyridine (30.91), 7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione (23.97), 2-Methyl-6-Styrylpyridine-3,4-Dicarboxylic Acid (26.62),

Methyl 3-(pentafluoro phenyl)acrylate (32.14), 2-Methyl-7-nonadecene (21.36), 1-Phenyl-1-(1'-naphthyl)-1,2-hexadiene (22.99). The GC-MS chromatogram is shown in the figure 1 and the characterized compounds were shown in table 2.

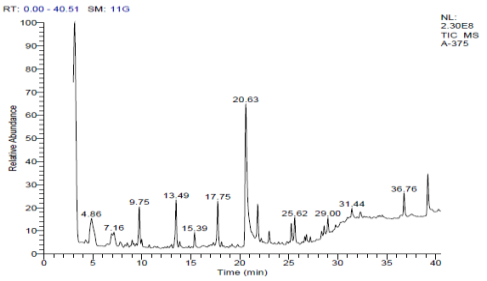

Figure 1: GC-MS chromatogram of ethanol extract of *Wrightia tinctoria* leaves

Table 2: Chemical composition of ethanol extract of *Wrightia tinctoria* leaves

S.NO	COMPOUND NAME	RETENTION TIME	AREA
1.	3-Dodecen-1-al	20.42	2.35
2.	2-Methyl-7-nonadecene	21.36	2.03
3.	1-Phenyl-1-(1'-naphthyl)-1,2-hexadiene	22.99	2.88
4.	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	23.97	3.90
5.	6-Amino-5-benzylamino-3-methylsulfonyl-1,2,4-triazine-1-oxide	25.98	3.30
6.	N-Ethyl-5,8-difluoro-1,4-dimethyl-1,2,3,4-tetrahydropyrido[3,4-b]pyrazin-7-amine	26.35	3.06
7.	2-Methyl-6-Styrylpyridine-3,4-Dicarboxylic Acid	26.62	1.24
8.	6-{[(1',1'Dimethylethyl) dimethylsilyl]oxy]-à-4-[(phenyl methoxy) phenyl]naphthalene-2-methanol	29.96	1.01
9.	2-Ethyl-5-propylpyridine	30.91	4.23
10.	4-(4-Chlorobenzoyl)-1-cyclohexyl-5-tosylamino-1H-1,2,3-triazole	31.53	4.68
11.	Dimethyl-2-[(ethoxycarbonyl)(3,4-dihydro-2H-1,4-benzothiazin-3-ylidene)methyl] fumate	31.83	3.39
12.	Methyl 3-(pentafluoro phenyl)acrylate	32.14	1.87
13.	5,5"-Dibromo-4'-methoxymethoxymethyl-2,2':6',2"-terpyridine	34.28	6.31
14.	2,5-Dimethoxybenzaldehyde oxime	37.52	9.52
15.	[4'-(3"-Ethyl-5"-methyl-2"-pyrrolyl)-2',3'-dichlorophenoxy]-acetic acid	38.23	2.02
16.	Methyl 2,12-Dibromo-7-phenyl-5,6,8,9-tetrahydrobenz[a,j]-anthracene-14-carboxylate	39.47	1.25
17.	(3-Fluoropyridin-2-yl)-[5-hydroxy-1-(4-methoxybenzyl)-1H-pyrazol-4-yl]methanone	39.88	1.91
18.	Ethyl-1[(5,6-difluoro-1-methyl-2-benzdiazolyl)amino]-6,7,8,9-tetrafluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate	40.25	1.06

In vitro anticancer activity by MTT assay

MTT assay is a reliable method for screening the anticancer activity by measuring the cell viability. The present study examined the *in vitro* anticancer activity of ethanol extract of *W. tinctoria* leaves against NH40 cell line using MTT assay. The incubation with different concentration of the extract affected the viability of NH40 cell line in dose dependent pattern. The extract was diluted to 5µl, 25µl, 50µl, 75µl, 100µl. The cell viability of NH40 cancer cells after incubation in different concentration of ethanol extract of leaves of *W. tinctoria* is depicted in figure 2. The IC_{50} value was 100µl and results showed that the ethanol extract posses significant anticancer activity.

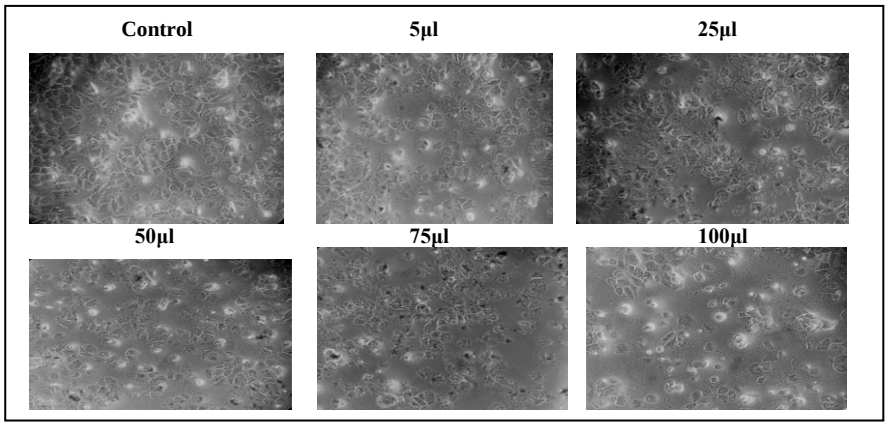

Figure 2: The cell viability of NH40 cancer cells after incubation in different concentrations of ethanol extract of leaves of *W. tinctoria.*

Table 3: % Cell inhibition in different concentration of ethanol extract of leaves of *W.tinctoria*.

S.No	Concentration (µl)	% Cell inhibition
1.	5	15
2.	25	21
3.	50	34
4.	75	39
5.	100	45

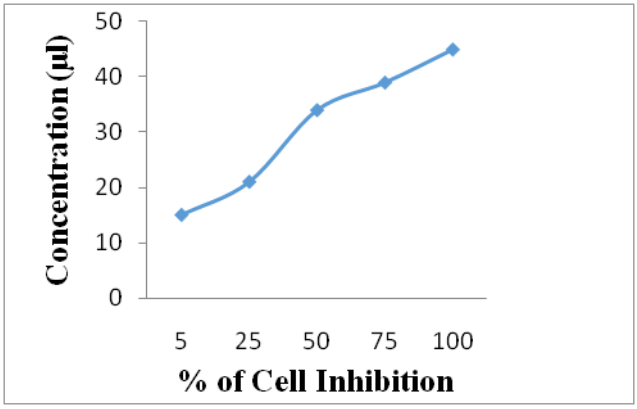

Figure 3: The IC_{50} value is determined by the plot of concentration Vs % incubation

IV. Conclusion

The present study reveals that the ethanol extract of *Wrightia tinctoria* leaves has shows that slight anticancer activity against NH40 Cell line. The isolation of active phytoconstituents is under process.

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