



PHYTOCHEMICAL AND PHARMACOLOGICAL ACTIVITY OF CAFFEYOYLQUINIC ACID FROM SEEDS OF *WILAYATHI-TULSI*

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ABSTRACT

Hyptis suaveolens (L) Poit Commonly known as wilayati tulsi, has a place with the family Lamiaceae. The current examination manages the Anti-disease and primer phytochemical screening on the *Hyptis suaveolens* (L) Poit. The plant is energizer, Carminative, Anticancer, Antirheumatic, Antimicrobial, Antidiabetic. Starter phytochemical screening uncovered the nearness of Volatile oil, alkaloid, glycoside, saponin, tannin and flavonoids and so on. Tender loving care was performed to recognize the mixes present in ethanolic concentrate of seeds. IR Spectroscopy gauges the vibrations of molecules and dependent on this it is conceivable to decide the useful gatherings. The unrefined ethanolic concentrate of seeds of *Hyptis suaveolens* was assessed for its Anti-disease action. These concentrate were researched for cytotoxic impact and apoptotic cell demise enlistment in human T leukemia cell line, Jurkat cells. This can be additionally read and clinically created for immunomodulative operators and disease preventive and remedial medications.

INTRODUCTION

Conventional medication is the most established type of social insurance on the planet and has been utilized in the anticipation and treatment of different sorts of sicknesses. Truly, various social orders have created different valuable recuperating strategies to battle wellbeing and perilous diseases^{1, 2}. The Restorative plants are the foundation of customary medication, where more than 3.3 billion individuals in the less evolved nations use on a standard basis³. In addition, WHO assessed that over 80% of the total populace despite everything depends on conventional medication for their essential social insurance needs⁴. It is additionally essential to take note of that WHO underlined the significance of customary medication in the wellbeing framework, and made methodologies, rules and measures for organic medicines⁵. Restorative Plants are restricted to conventional utilization as well as considered as a rich wellspring of fixings in the

Improvement of the cutting edge drugs⁶. For instance, the disclosure of current medications, for example, quinine, vincristine, digoxin and digitoxin, artemisinin, and so forth, from restorative plants connotes the tremendous possible that despite everything exists for the creation of a lot progressively novel pharmaceuticals⁷. *Hyptis suaveolens* (L.) Poit. is a plant having a place with family Lamiaceae, or the Mint family. It is a group of plants of around 210 genera and somewhere in the range of 3,500 species⁸. The lamiaceae can be perceived as any herb or bush having square stems, inverse leaves, and emitting a minty smell. They incorporate various restorative and sub-therapeutic plants of incredible worth. This significant request has no harmful individuals. They for the most part show fragrant or unpleasant sweet-smelling, energizer and astringent properties. They are utilized as tonics, emmenagogues, diaphoretics and against spasmodics. The leaves of numerous individuals

contain fragrant fundamental oils⁹. The nonexclusive name Hyptis has been gotten from Greek word Hyptis is "laid back or resupinate", alluding to the appendage of the corolla, which is turned on its back¹⁰. It is a huge variety of around 400 species and is local of warm tropical America. Various species have gotten naturalized in the hotter pieces of the old world. A few animal types have been accounted for to have therapeutic properties. The variety is set apart by diaphoretic, carminative, antifungal, antibacterial, anticonvulsant and vulvery properties and is as often as possible detailed in the treatment of gastrointestinal diseases, to ease spasms and agony, just as in the treatment of jungle fever. Four species are found in India¹¹. Hyptis suaveolens is a typical plant in India. The plant might be gathered in enormous amounts from the wild just as from those refined as a yield by the Indians. Indians used to call it "Chan/Wilayathi tulsi" and the morning soup made by blending it in with corn is designated "Bate" which implies memory help. The plant is a sweet smelling woody herb with tetragonal hispid stem, leaves long petioled, pubescent, extensively elliptic-praise, base angled, shorten or intense, edge coarsely serrulate, peak intense, chartaceous. It is ordinarily found in thick bunches on street sides, corrupted wet and dry deciduous woodland, squander lands and over-touched field.

MATERIALS AND METHODS:



Figure.1. *Hyptis suaveolens* plant and seeds

TAXONOMICAL STUDY OF *HYPTIS SUAVEOLENS*

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Sub phylum: Angiospermae

Class: Dicotyledonae

Order: Lamiales

Family: Lamiaceae

Genus: Hyptis

Species: *Hyptis Suaveolens*(L.)Poit.

Synonym: *Ballotasuaveolens* L.

Common Name: Wilayathi-Tulsi

Plant Materials:

The plant seeds of *Hyptis suaveolens* (Figure no.1) are gathered from Sri Krishnadevaraya College, Ananthapuramu, 515003, A.P., India, in the long stretch of Spring and verified by Dr. B. Ravi Prasad Rao, Professor, Biodiversity conservation Division, Department of botany, the plant voucher number 57405(SKU). The plant seeds materials were washed altogether for 3-4 times with running faucet water to expel dust from the surface and twice with clean refined water, conceal dried at room temperature on a clean smudging paper, after complete drying the plant seed materials were powdered utilizing the blender and put away in independent water/air proof containers at that point utilized for the arrangement of ethanol extricates.

Preparation of extracts:

Dried and coarsely powdered seeds (500 gm,) of *Hyptis suaveolens* were exposed to extraction in Clevengers contraction extractor utilizing ethanol was showed in Figure no. 2. The concentrates of seeds were concentrated by vacuum refining and afterward dried in open air¹².

Qualitative phytochemical evaluation:

The various qualitative chemical tests were performed for establishing the profile of the aromatic plant *H. suaveolens* extracts for its chemical composition.



Figure 2: Extraction of *Hyptis suaveolens*

Qualitative phytochemical analyses were done by using the procedures of Kokate (1994)¹³ and Kokate *et al.*, (1995)¹⁴ Sofowara (1993)¹⁵, Alkaloids, Flavonones, Terpenoids, Steroids, Tannins, Saponins, Anthraquinones, Aldehydes, Ketones.

CHEMICAL CONSTITUENTS¹⁶:

- The phytochemical constituent of the plants are as follows: alkaloid (0.02- 0.07 mg ml⁻¹), flavonoid (0.05- 0.12 mg ml⁻¹), phenol (0.077- 0.978 mg ml⁻¹), saponin (0.04- 0.08 mg ml⁻¹) and tannin (0.283- 0.982 mg ml⁻¹).
- *Hyptis suaveolens* seed oil (Yield: 17.44 %) contained linoleic acid (76.13 %), oleic acid (10.83 %), palmitic acid (6.55%), stearic acid (4.56%) and heptacosanoic acid (1.94%) as the major constituents.

ANTI-CANCER ACTIVITY:

Anti-Cancer Activity carried by MTT assay

Principle of Assay:

This is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, coloured (dark purple) formazan product. The cells are then solubilised with an organic solvent (eg. DMSO, Isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reductions of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells.

Materials

MOT [Leukemia (T Lymphocyte)]:

Dulbecco's Modified Eagle Media (DMEM) with low glucose -Cat No-11965-092 (Gibco, Invitrogen) Fetal bovine serum (FBS) - Cat No -10270106 (Gibco, Invitrogen) Antibiotic - Antimycotic 100X solution (Thermofisher Scientific)-Cat No-15240062. Figure .4,5.

Protocol

Cytotoxicity:

The cells were seeded a 96-well flat-bottom micro plate and maintained at 37°C in 95% humidity and 5% CO₂ for overnight. Different concentration (400, 200, 100, 50, 25, 12.5 µg/ml) of samples were treated. The cells were incubated for another 48 hours. The wells were washed twice with PBS and 20 µL of the MTT staining solution was added to each well and plate was incubated at 37°C. After 4h, 100 µL of DMSO was added to each well to dissolve the formazan crystals, and absorbance was recorded with a 570 nm using micro plate reader.

Formula:

Surviving cells (%) = Mean OD of test compound / Mean OD of Negative control × 100

Using graph Pad Prism Version 5.1, we calculate the IC₅₀ of compounds.

RESULTS & DISCUSSION:

Preliminary Qualitative Tests of Various Extracts of *Hyptis suaveolens*:

DISCUSSION:

In the presence of study, dried power seeds of *Hyptis suaveolens* were subjected to extraction using ethanol as solvent using Clevenger's process. Some part of extracts was reserved for preliminary phytochemical investigation and rest was utilized for phytochemical screening and pharmacological activity. The Preliminary Phytochemical investigation showed presence of Volatile oil, Alkaloids, Glycosides, Tannins, Saponins and flavonoids compounds.

Table no 1. Preliminary qualitative tests of various extracts of Hyptissuaveolens

Plant Extract	Ethanolic extract of H. suaveolens
Volatile oil	+
Alkaloids	+
Glycosides	+
Steroids	-
Gums	-
Tannins	+
Saponins	+
Flavonoids	+

THIN LAYER CHROMATOGRAPHY

Rf values of active constituents by TLC

Table no .2.Rf values of active constituents by TLC

Solvents are Toluene: Ethyl acetate (9.3:0.7)	
Hyptissuaveolens seed extract	RfValues
Sample value	0.406

IR SPECTROSCOPY:

Table no.3. IR spectra values

S.NO	Functional groups	Observed value	Standard value
1	C-H	2906.17	2850-2970
2	COOH	1761.12	1690-1760
3	Aromatic Group	1644.08	1500-1600
4	OH	3419.78	3200-3600
5	C=C	2980.35	3010-3095

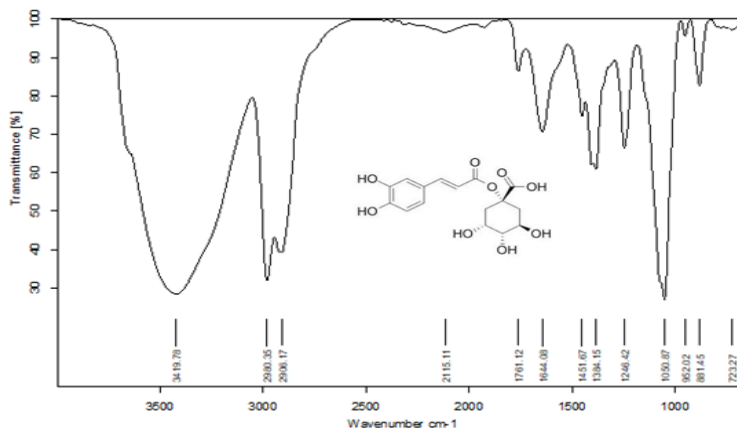


Figure.4. Graph of IR spectroscopy

ANTI-CANCER ACTIVITY:

Formula: Surviving cells (%) = Mean OD of test compound / Mean OD of Negative control × 100
Using graph Pad Prism Version5.1, we calculate the IC 50 of compounds

Results:

Table no .4 .IC50 VALUE OF COUMPOUNDS IN µl/ml

Sample Code	MOT cells
CA	45.83
Paclitaxel	247.6327
	0.29 µM

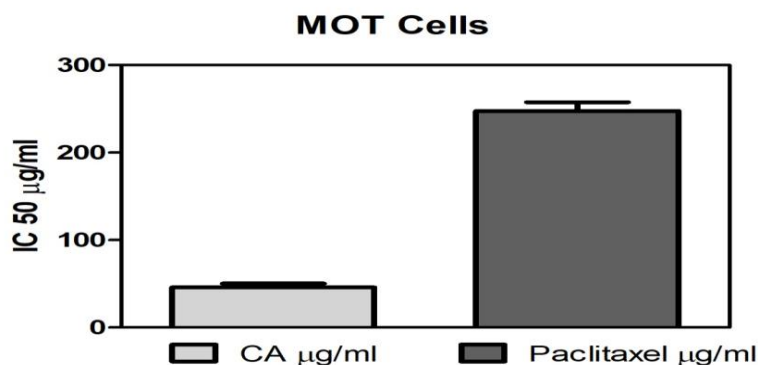


Figure: 4 - Graph for IC50 VALUE OF COUMPOUNDS IN µl/ml
Table no .5. Mean Cell Viability (CA vs MOT)

Concentration µl/ml	CA
400	20.69
200	28.16
100	30.38
50	80.38
25	91.63
12.5	94.91
Negative control	100

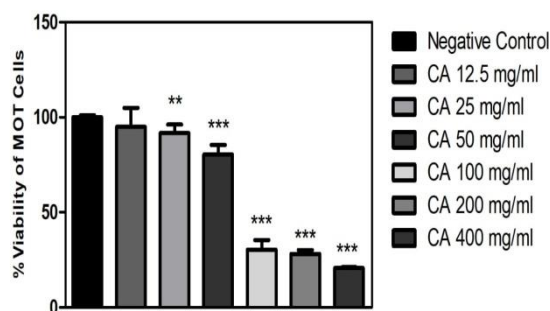


Figure .5Graph for Mean Cell Viability (CA vs MOT)

The Volatile oil compound being the major Phyto chemical constituent was isolated from aqueous extract of seeds and subjected to qualitative TLC analysis, IR Spectroscopy. Isolation the constituents using chromatographic methods (TLC) by using mobile phase in the ratio of 9.3:0.7 (Toluene: Ethyl acetate)IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups. Investigated *in-vitro* evaluation of anti cancer activity of *Hyptissuaveolens* plant seeds aqueous crude extract in living tissues

CONCLUSION:

Based on the current investigation, the outcomes at long last inferred that the ethanolic concentrate of *Hyptissuaveolens* seeds have against disease movement. These action might be because of the solid event of polyphenolic mixes, for example, Volatile oil, Alkaloids, Glycosides, Tannins, Saponins and flavonoids. This exploration gives a thought that the compound of the plant *Hyptissuaveolens* can be utilized as lead compound for structuring powerful medications which can be utilized for treatment of different sicknesses, for example, disease and different issue. Anyway these examinations are not

adequate to guarantee and consequently different other pharmacological, phytochemical and bio systematic investigations followed by observational investigations in people are to be completed so as to help customary significance of the expressed exercises.

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