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STANDARDIZATION & PHARMACOLOGICAL EVALUATION OF POLYHERBAL EXTRACT TO REPORT THE ANTICARCINOGENIC POTENTIAL

Shubham Burman [1], Bhuwanendra Singh [*2], Arvind Kumar [1] and Vaishali [1]

Department of Pharmaceutical Chemistry [1], Department of Pharmacognosy [2], S. D. College of Pharmacy & Vocational Studies, Muzaffarnagar - 251001, Uttar Pradesh, India.

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Correspondence to Author:
Dr. Bhuwanendra Singh

Professor,
Department of Pharmaceutical Chemistry, S. D. College of Pharmacy & Vocational Studies, Muzaffarnagar - 251001, Uttar Pradesh, India.

E-mail: bhuwanendrasingh14@gmail.com

ABSTRACT: The relevance of traditional medicines and their folkloric applications for the well-being of humanity have been highlighted in the current study. To generate the polyherbal extract for the *in-vitro* assessment of anticancer activity, the researcher has investigated the standardization criteria for the individual plant drug and extract obtained from these herbs. According to the literature and earlier studies, all four of the herbs utilized to create polyherbal extract have the potential to cure a variety of malignancies in addition to liver carcinoma. The physiochemical and pharmacological value of each medicinal herb and the polyherbal extract of these four plant drugs (Vinca, Jackfruit, Lawsonia, and Ashwagandha) are revealed in the same research findings. Based on the results of phytochemical testing, it is evident that the majority of these phytochemicals employed include the alkaloids and steroids in n-hexane extract, which also suggests the possible anticancer effects of each drug separately. The MTT dye assay assessed the polyherbal extract's cytotoxic impact on the Huh7 cell line. The polyherbal extract's anticancer results are highly remarkable. For the foreseeable future, it may be possible to assess the *in-vivo* cancer Activity directly on the chosen animals. Some specific formulations may be created using the potent crude drug extract created by selecting these powerful anticancer plant drugs.

INTRODUCTION: The traditional Indian medical system known as Ayurveda is still among the oldest extant traditions with a strong analytical & exploratory foundation. That is the discipline of living that uses individualized treatment and an integrated talk to healthiness. The role of Pharmacognosy is also very important in obtaining information on crude drugs. Ayurveda, often known as the discipline of self-healing, holds that every cell is innately a necessary representation of pure intellect [1]. In this conventional Indian school of medicament, herbal treatments are equally significant to the self-healing idea.

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Ayurveda; its primary focus was therapeutics. The Charaka Samhita included a list of 341 plants and plant products for use in medicine [2]. The next landmark in Ayurvedic literature was the Sushruta Samhita (600 B. C.) [3]. Herbal formulations are made using plant extracts, whole plant material, and the proper excipients. These extracts include active ingredients; thus, different formulations are created and standardized using the appropriate excipients or bases.

Polyherbal formulations are those that contain two or more herbs. It is highly advised to do further cellular and molecular investigations to clarify the processes behind the anticancer effects of herbs. To do this, to evaluate the effectiveness of natural anticancer medicines, numerous *in-vitro* and *in-vivo* approaches have been created. Examples of *in-vitro* methods include the Tryphan blue dye exclusion assay, the LDH (Lactic dehydrogenase) assay, and the MTT assay.

Description of Plant Drug Used Vinca, Jackfruit, Lawsonia, Ashwagandha: Vinca is also known as Pervincal Mill, Sadabahar, and Periwinkle are common names for Vinca, which is a member of the Apocynaceae family of the Plantae kingdom [4]. Vinca is members of the Tracheophyta division of the class Magnoloipsida. Artocarpus integrafolius, sometimes known as Jackfruit, is a synonym for this fruit [5, 6]. It also goes by the common names Kathal in Hindi and Jackfruit in English. Tracheophytes, which include jackfruit, make up this class. Synonyms of *Lawsonia*is *Alcanna spinosa,* & its common name in Hindi Mehandi & English name Hennawhich belong to the family Lythraceae and its kingdom Plantae. *Lawsonia* are from the class of Dicotyledos. Synonyms of *Ashwandgha is Vajikari*, & its comman name in Hindi *Ashwandgha* & the English name Winter cherry, Jackfruit, which belong to the family Solanaceae and its kingdom Plantae. *Ashwandgha* are from the class of Magnoliopsida [7].

MATERIALS & METHODS:

Collection, Identification & Confirmation of Plant Material: Vinca, Lawsonia, Ashwagandha, and Jackfruit were the plants employed in this experiment. They were all purchased locally from a Muzaffarnagar, Uttar Pradesh, India, nursery. The plant sample was accurately identified and taxonomy validated by Dr. Salik Noorani of the Department of Botany at Jamia Hamdard University in New Delhi. The institute was given the voucher specimen [BOT/DAC/Nov, 2021/07] for future use.

Preparation of Plant Material by using Polyherbs: Before being pounded into powder and pushed through a sieve (lattice dimensions # 40), the plant material (Leaves) was collected separately and left to dry for about five to six days in the shade. Then 500 grammes of each powder medication were combined. To identify the plant metabolites, the same powder was then utilised.

Determination of Foreign Matter: 50 grams of the drug sample were weighed and carefully stacked. An unaided eye examination revealed the alien object. We determined the present proportion after separating, weighing, and sifting it. The study's medications lacked debris, rocks, insects, animal faeces, and other toxins like mould and insects [8].

Sensory & Botanical Characters: Taste was reported based on a variety of academic sources, while touch, texture, and other botanic qualities including scent and colour were evaluated individually with the use of a magnifying lens.

Physiochemical Evaluation for the Standardisation of Crude Drug [9]:

Identifying Extraneous Stuff: A drug sample weighing 50 g was spread thinly. The foreign material was discovered after a visual inspection. After that, it was divided and weighed, and the proportion of it present was estimated. The pharmaceuticals that were submitted for additional inspection were free of impurities, including soil, stones, insects, animal faeces, and mould [8].

Moisture Content Measurement Using a Hot Air Oven: The method described below was used to estimate the moisture content of a substance that looked to contain just water as a volatile ingredient. A tare evaporation plate held 2.78 grams of medication that had been accurately measured (without prior drying). The percent was calculated using the inceptive mass after five hours of drying the medicines at 105 degrees Celsius [9].

Determination of Ash:

Determination of Total Ash: A silica dish bowl was used to fire about two grams of the medications in powder form at a temperature of no higher than 450 degrees Celsius until no free from carbon was left. After cooling, the finished mass was measured. Utilising the air-dried medicine as a reference, the calculated ash percentage [10].

Assessment of Acid Insoluble Ash: After being cleared of ash by a hot flood and being burned with 25 milliliters of mild hydrochloric acid to a constant mass after 5 minutes of boiling, ash is deposited on a membrane filter. Evaluating the ratio of the measurement-allowed amount of acid-insoluble ash air-dried to the medication [10].

Determination of Water-soluble Ash: Insoluble wastes were collected on ashless filter paper; the ash was warmed in 25 cc of water for 5 minutes, rinsed with lukewarm water, and burnt at 450°C. After subtracting the insoluble's weight, the pharmaceutical ash's mass was determined. Different ash and moisture calculations are made

by weight. Last, the amount of water-based ash was calculated using a medicine that freezes-dries [10].

Identifying Extractable Material:

Polyherbal Extract: To increase the combined efficacy of many drugs, polyherbal extracts are employed in polyherbal formulations, which have more pharmacological potential and fewer adverse effects.

Procedure: To create the polyherbal (Phf), the four herbal medicines Vinca (leaves), Lawsonia (leaves), Ashwagandha (leaves), and Jackfruit (leaves) were all chopped and reduced to a coarse powder after being properly dried individually, and this coarse powder was then combined to create the polyherbal extract.

Process 1:

Hot Extraction (Successive): For refluxing, around 25 g of coarsely powdered, air-dried, crude drugs were employed, each in a thimble, with various solvents such as hexane, ethyl acetate, & alcohol (Ethanol). The ratio of extractable is then estimated after recovery solutions under vacuum and drying in desiccators. All four medicines were combined and extracted with the same solvents simultaneously to create a polyherbal extract.

Process 2:

Cold Maceration: Two grams or more of coarsely powdered, air-dried material were weighed in an Erlenmeyer flask with a bottomed flask. It was then permitted to stand for 18 hours and rapidly filtered to avoid solvent loss after being agitated regularly for 6 hours with 100 mL of solvents, including hexane, ethyl acetate, alcohol (Ethanol), & water. Each drug was used separately to obtain the crude extract, and at the same time, all four drugs were mixed and extracted with the same solvents to obtain the polyherbal extract. The extracted material was dried at 105 degrees Celsius for six hours and chilled for thirty minutes in desiccators [11].

Pharmacological / Therapeutical Parameters:

Determination of Swelling Index: A graduated cylinder with a glass stopper and a capacity of 25 ml was filled with an accurate weight of around one gramme of fine powder. The cylinder's inner diameter was around 16 mm, and the stepped portion's length, which went up in 0.2 mL increments from 0 to 25 Ml, was roughly 12.5 mm. Every 10 minutes, the mixture was thoroughly stirred and bubbled with 25 cc of water. The quantity of plant powdered medicine in the mixture, including any sticky mucilage, has been quantified to within milliliters after three hours of storage at room temperature. The average value of each measurement was computed from one gram of plant material [11].

Determination of Foaming Index: Approximately 1.0 g of drug powder that has been coarsely milled has been added to a 500 mL Erlenmeyer flask that has 100 mL of hot water in it. Thirty minutes have passed with a moderate boil being maintained. Pour distilled water into a 100 mL graduated flask after it has been chilled and filtered. The soup was poured into 10 sample test tubes with stoppers that were marked with 1 ml, 2 ml, 3 ml, *etc* (16-cm length & 16-mm circumference). Then, add water to the halfway point in each tube, up to 10ml. The test tube caps were left on and shaken at a rate of two shakes per second for 15 seconds into a longitudinal wave. After waiting 15 minutes, the foam's height was measured. Results are summarised as follows [11]. They reach a foam index below 100 when they surf at a height within each subsequent one-cm pipe. When each tube has a foamy top and one cm of material within, the index is determined using the concentration of plant matter inside the pipe. Produce an interim infusion to get a more exact result, whether it's the tube's first or second lumen row. For each pipe, it foams more than index one thousand when they foam at a height larger than 1 cm. In order to get the desired outcome, they need to repeat the test using a fresh set of decoction dilutions.

Measure the foam the index using method below:

$= 1000 / a$

Where a denotes the volume of decoction (in millilitres) required to create a dilution in a tube that froths to a height of one centimetre.

Preliminary Phytochemical Investigation of the Crude Drug Extracts: To examine the chemical groups found in the medications, preliminary phytochemical experiments have been conducted. Unless otherwise noted in the relevant assay, a 10% (w/v) extract solution was employed. The findings

of the chemical group test are now available in tabular form. A standard screening approach was used to qualitatively identify the categories of organic chemicals detected in different plant extracts [12].

Alkaloids: By dissolving a few milligrammes of extracts in five millilitres of filtered water, adding two millilitres of dilute hydrochloric acid (HCL) before the reaction starts, and then adding one milliliter of Dragendorff's reagent, the Dragendorff's test generates an orange or orange-red ppt fairly quickly.

Hager's Test: Put 1 ml of the drug's extract and a little quantity of Hager's reagent into a test tube. Alkaloids are present because a yellowish precipitate has developed.

Wagner's Test: To perform the test, combine one millilitre of the drug's extracts with a little quantity of Wagner's reagent, then acidify while adding 2% volume/volume HCL. The Ppt becomes brown or yellow.

Mayer's Reagent: To 1 ml of the drug's extract, add a little quantity of Mayer reagent in light yellow or colourless.

Carbohydrates: Extracts from the medicine in a ratio of 0.5 mL to 2 mL of the stool sample, solun Carbohydrates can be separated based on whether they are green or blue in colour. Benedict's test involved boiling a solution for up to five minutes after adding five millilitres of Benedict's solution to five millilitres of powdered drug and alcohol extract. A brick-red precipitate formed with the addition of carbohydrates.

Fehling Test: One millilitre of Fehling solution ('A' & ('B') mixed with two millilitres of the plant's crude medicine extract were then boiled for a short time. A reddish-brown PowerPoint is the outcome.

Molisch's Test: 2 ml of concentrated sulfuric acid was added to 2 ml of extracts. When too much alkaline solution is added, the red-violet ring that forms when carbs are present disappears.

Flavonoid:
Shinoda Test: Add five to ten drops of mild HCl and a few pieces of Mg to the sample tube that contains a half millilitre of a hydroalcoholic crude powder drug extract. A pinkish, red-pink, or brownish hue results from flavonoids.

Triterpenoid: A technique for determining whether triterpenoids are present is the Liebermann-Burchard assessment. 2 millilitres of acetic anhydride solution, 1 millilitre of con sulphuric acid, and 1 litre of alcohol-based drug extracts in chloroform. The development of a violet ring serves as a marker for triterpenoids.

Saponins: Add a few drops of $NaHCO_3$ solution to a sample tube with an extract of the medicine and shake vigorously for three minutes. The foam resembles a honeycomb.

Steroids: One millilitre of the plant extracts in methanol, two millilitres of acetic anhydride solution, and one millilitre of conc. sulphuric acid before becoming blue, it acquires a greenish colour. Salkowaski's response: One millilitre of concentrated H2SO4 should be carefully added to two millilitres of alcohol drug extracts from the sample tube side. A crimson colour appears on the chloroform layer.

Tannins: A 5% ferric chloride solution was added to one to two millilitres of crude powdered drug extracts. Brown tannin has a brown colour, while gallotannin is greenish.

Starch: After combining 0.015 g I2 and 0.075 g KI (potassium iodide) in 5 mL of sterilised water, add 2-3 mL of medicinal plant extracts. It causes the colour blue to appear.

Proteins: Add five to eight drops consanguineous to a 10% weight/volume sodium hydroxide solution and one or two drops of a 3% weight/volume $CuSO_4$ solution to one millilitre of the medicinal substance extract. Its colour is reddish-violet.

Million Tests: Mix a little quantity of the drug's extract with water. 1 cc of distilled water is mixed with five to six drops of Million regent. A white Power Point slideshow becomes crimson when heated.

Fluorescence Analysis of the Powder Drug & Various Extracts: The therapeutic drug's fluorescence was identified in both UV and daytime using various solvent extracts (254nm).

The powder was exposed to neutral solvents including hexane, benzene, chloroform, alcohol, water, together with acetone, alkaline solutions like aqueous and alcoholic 1N NAOH, as well as acids like 1n HCL and 50% H_2SO_4 [13, 14,15].

Biological Evaluation of Extract Obtained from:
Anticancer Potential of Polyherbal Formulation (Hepatocellular Carcinoma):
Materials: 96 well plates, a micropipette, culture media, a negative control, a vortex shaker, and other materials are included in the MTT solution and kit.

Procedure:
Hepatic Cancer Cell Line Tests for *In-vitro* Cell Viability: The cytotoxic effect of the polyherbal extract on the Huh7 cell line was evaluated using the MTT dye test. The sole medium used in the control testing was the whole growth culture media (serving as non-toxic control). In a 96-well plate with the growth culture media, three cell types (100 L) were sown at a density of 6 104 cells/well. The medium was replaced with 200 mL of media with different concentrations of extracted polyherbal extracts (5, 10, 20, 30, 40, and 50 g/mL) after a 12 hour culture period. Each well's growth medium was taken out after a set amount of time, and the cells went through two rounds of washing in 1 PBS. Then, each well was filled with 20 L of the MTT solution (5 mg/mL in PBS, pH 7.4) and 200 L of the growing culture material. The formazan crystals were first dissolved in 150 L of DMSO for 10 minutes after the medium had been incubated for 4 hours at 37 °C with 5% CO_2. A micro-scan spectrum was used to calculate the amount of formazan from the optical density at 570 nm (Thermo Scientific,). In compared to the non-toxic control, the findings were displayed as percentages. The findings are presented in the result and discussion chapter

RESULT & DISCUSSION:

Examination for Presence/Absence Regarding Foreign Matter: To assure the purity of the plant material used in the research, foreign materials must be identified. After this study, it was found that the used herbal material was free of all contaminants and adulterants, including *Vinca* (Leaves), *Lawsonia* (Leaves), *Ashwangandha* (Leaves), and *Jackfruit* (Leaves) in the polyherbal powder form. **Table 1** display the test results. So, it was determined that neither the plant medication powder taken individually nor the polyherbspowder created from it had any impurities. We can thus draw the conclusion that this test parameter is extremely important both in the starting stage and in the end stage of the process to assure the quality of the employed drug material.

TABLE 1: SHOWING THE RESULT REGARDING DETERMINATION OF FOREIGN MATERIAL

Powdered Drug	Parameters	Observations
Vinca	Foreign Organic matter	No Adulterants
Lawsonia	Foreign Organic matter	No Adulterants
Ashwangandha	Foreign Organic matter	No Adulterants
Jackfruit	Foreign Organic matter	No Adulterants
Polyherbs powder	Foreign Organic matter	No Adulterants

Sensory & Botanical Characters (Morphology): Evaluation of each drug independently was crucial for identifying plant drugs, and confirmation was done based on visual experience and with the aid of several literature surveys on their colour, odour, and texture. The results are shown in **Table 2.** Therefore, this examination has been extremely beneficial in choosing real herbal medicines.

TABLE 2: SENSORY BOTANICAL & CHARACTERISTICS OF THE DRUGS USED

S. no.	Plant Drugs (Leaves)	Characters	Observations
1.	*Vinca*	Touch/Texture	Smooth
		Odour	Characteristic
		Taste	Bitter
		Colour	Green
2.	*Lawsonia*	Touch/Texture	Smooth
		Odour	Characteristic
		Taste	Bitter
		Colour	DarkGreen/Green Brown/Dull Green

3.	*Ashwagandha*	Touch/Texture	Slightly rough
		Odour	Like horse Urine/strong smell of green tomatoes
		Taste	Bitter
		Colour	Dull green
4.	*Jackfruit*	Touch/Texture	Leathery
		Odour	Cloying sweet
		Taste	Bitter
		Colour	Dark Green

Physiochemical Analysis for All four Herbs *Viz: Vinca, Lawsonia, Ashwagandha, Jackfruit* (Leaves): The World Health Organization's criteria were followed, and each test parameter's physical and chemical restrictions or specifications were followed. In order to guarantee the quality, purity, and effectiveness of herbal medicines and plant-based products, the WHO offers a variety of criteria for evaluation. Although not all of the test parameters needed by the WHO are applicable to all medicinal plant materials, the maximum test parameters should be finished to report on the efficacy of the utilised tree medicine. The substance's phytochemistry and pharmacological qualities were investigated at the same time as a survey of the literature. Some of the many physiochemical and pharmacological criteria used include the presence or absence of water or moisture, the number of different types of ashes, the capacity to expand and form foam, and the fluorescence evaluation of powdered medications. **Table 3** displays the results and computed values and thoroughly explains each parameter. This emphasizes how important testing is in determining the effectiveness and purity of the medication used in the current study.

TABLE 3: RESULTS OF PHYSIOCHEMICAL ANALYSIS OF ALL FOUR DRUGS & POLYHERBAL POWDER

S. no.	Parameters	Observations
	1 Moisture Content *(%w/w)*	
1. Vinca	Moisture Content *(%w/w)*	5.08
2. Lawsonia	Moisture Content *(%w/w)*	3.5
3. Ashwagandha	Moisture Content *(%w/w)*	2.0
4. Jackfruit	Moisture Content *(%w/w)*	1.04
5. Polyherbal powder	Moisture Content *(%w/w)*	1.02
	2 Physicochemical Parameters	
	Ash Values (% w/w)	
1. Vinca	(A) Value of total ash	2.5
	(B) Value of acid-insoluble ash	1.69
	(C) water-soluble ash	1.78
2. Lawsonia	(a) Value of total ash	8.0
	(b) Value of Acid Insoluble Ash	4.40
	(c) Water Soluble Ash	3.99
3. Ashwagandha	(a) Value of Total Ash	8.99
	(b) Value of Acid Insoluble Ash	5.88
	(c) Water Soluble Ash	6.77
4. Jackfruit	(a) Value of Total Ash	9.0
	(b) Value of Acid Insoluble Ash	12.1
	(c) Water Soluble Ash	13.67
5. *Polyherbal powder*	(a) Value of Total Ash	13.21
	(b) Value of Acid Insoluble Ash	7.88
	(c) Water Soluble Ash	8.99
	Extractive Values (% w/w)	
1. *Vinca*	*Hot extraction method (Sucessesive)*	
	n-Hexane (% w/w)	2.33
	Ethyl acetate (% w/w)	2.67
	Ethanol extract (% w/w)	3.78
	Cold Extraction (Maceration) *(% w/w)*	
	n-Hexane (% w/w)	2.45
	Ethyl acetate (% w/w)	2.84
	Ethanol extract (% w/w)	3.45
	Water (% w/w)	2.11

Lawsonia	*Hot extraction method (Sucessesive)*	2.33
	n-Hexane (% w/w)	1.34
	Ethyl acetate (% w/w)	2.44
	Ethanol extract (% w/w)	3.45
	Cold Extraction (Maceration) *(% w/w)*	
	n-Hexane (% w/w)	1.22
	Ethyl acetate (% w/w)	2.33
	Ethanol extract (% w/w)	3.55
	Water (% w/w)	4.56
Ashwagandha	*Hot extraction method (Successive)*	
	n-Hexane (% w/w)	2.34
	Ethyl acetate (% w/w)	4.33
	Ethanol extract (% w/w)	5.66
	Cold Extraction (Maceration) *(% w/w)*	
	n-Hexane (% w/w)	3.44
	Ethyl acetate (% w/w)	3.47
	Water	5.78
Jackfruit	*Hot extraction method (Sucessesive)*	
	n-Hexane (% w/w)	2.23
	Ethyl acetate (% w/w)	3.21
	Ethanol extract (% w/w)	4.66
	Cold Extraction (Maceration) *(% w/w)*	
	n-Hexane (% w/w)	3.23
	Ethyl acetate (% w/w)	5.21
	Ethanol extract (% w/w)	7.88
	Water	8.99
Polyherbal Powder	*Hot extraction method (Sucessesive)*	
	n-Hexane (% w/w)	5.66
	Ethyl acetate (% w/w)	6.44
	Ethanol extract (% w/w)	6.78
	Cold Extraction (Maceration) *(% w/w)*	
	n-Hexane (% w/w)	3.77
	Ethyl acetate (% w/w)	5.44
	Ethanol extract (% w/w)	6.77
	Water	9.88
3.	**Pharmacological/Therapeutic**	
Vinca	Swelling Index	1.5 ml
Vinca	Foaming Index	0.8 i.e., Less than 1 cm
Lawsonia	Swelling Index	2.5 ml
Lawsonia	Foaming Index	0.9 i.e., Less than 1 cm
Ashwagandha	Swelling Index	3.6 ml
Ashwagandha	Foaming Index	0.7 cm i.e., Less than 1
Jackfruit	Swelling Index	0.2 ml
Jackfruit	Foaming Index	0.8cm i.e., Less than 1
Polyherbal Powder	Swelling Index	1.7 ml
Polyherbal Powder	Foaming Index	0.8 cm i.e., Less than 1

DISCUSSION: There is little probability of any enzymatic or microbial breakdown in the plant medication as the medicinal ingredient includes very little moisture. When employing air-dried material, the moisture content had to be calculated and was found to be 5.08, 3.5, 2.0, 1.04, and 1.02% w/w for *Vinca, Lawsonia, Ashwagandha, Jackfruit, a*nd Polyherbal powder, respectively. The medicinal plant drugs have very little impurities in the form of carbon and inorganic, air-dried material, according to the current research. The researcher discovered a low and moderate amount of acid-insoluble ash with air-dried material in the current experiment, indicating very little sand and other silicious materials. Sand and other silicious materials can be detected by looking for acid-insoluble ash. During extraction, the concentration of the medication's active elements is assessed; the higher the extractive value, the more chemical components are present in the drug. The secondary metabolite was extracted in this case using a variety of organic solvents. The extraction was done by

increasing polarity by using different solvents. The highest value was found during hot extraction when water was used as the solvent, and the material was air-dried. At the same time, the value of cold maceration with air-dried material is greater in the water-soluble extract. Even though the water extractive values are higher than the ethanol extractive values, water extract is more prone to microbial growth than ethanol extract. To assess the biological potential of the medicines, ethanol extract was utilized. The swelling index, computed to evaluate the potency of plant medications employed in current research and to describe pharmacological effectiveness, also provides a clue as to whether the medication includes mucilage. The foaming index reveals the saponins' propensity to froth when a plant medicine has to be turned into an aqueous decoction.

Primary Screening for Phytochemicals (Secondary Plant Metabolites): Many different types of extracts from cold extraction processes are utilized to look for secondary metabolites. Alkaloids, glycosides, flavonoids, tannins, carbohydrates, terpenoids, and proteins are found in ethanol and chloroform. The outcomes are shown in **Table 4.**

TABLE 4: PHYTOCHEMICALS PRESENT IN THE AERIAL PARTS OF ALL THE DRUGS USED IN THE STUDY

S. no.	Drugs	Secondary Metabolites	Plant Drug Extracts Obtained from Cold Extraction Method in Different Solvents			
			n-Hexane	Ethyl acetate	Ethanol	Water
1		Alkaloids	RIP	RIP	RIP	RIP
2		Carbohydrates	RIA	RIA	RIP	RIP
3		Flavonoids	RIA	RIP	RIP	RIP
4		Triterpenoids	RIP	RIP	RIA	RIA
5		Saponins	RIA	RIA	RIP	RIP
6		Steroid	RIP	RIA	RIA	RIA
7		Tannins	RIA	RIA	RIP	RIP
8		Starch	RIA	RIA	RIP	RIP
9	*Vinca*	Proteins	RIA	RIA	RIA	RIA
10		Glycosides	RIA	RIP	RIP	RIP
1		Alkaloids	RIP	RIP	RIP	RIP
2		Carbohydrates	RIA	RIA	RIP	RIP
3		Flavonoids	RIP	RIP	RIP	RIP
4		Triterpenoids	RIP	RIP	RIA	RIA
5		Saponins	RIA	RIA	RIP	RIP
6		Steroid	RIP	RIA	RIA	RIA
7		Tannins	RIA	RIA	RIP	RIP
8	*Lawsonia*	Starch	RIA	RIA	RIP	RIP
9		Proteins	RIA	RIA	RIA	RIA
10		Glycosides	RIA	RIA	RIA	RIA
1		Alkaloids	RIP	RIP	RIP	RIP
2		Carbohydrates	RIA	RIA	RIP	RIP
3		Flavonoids	RIA	RIP	RIP	RIP
4		Triterpenoids	RIP	RIP	RIA	RIA
5		Saponins	RIP	RIP	RIP	RIP
6		Steroid	RIP	RIA	RIA	RIA
7		Tannins	RIA	RIA	RIP	RIP
8	*Jackfruit*	Starch	RIA	RIA	RIP	RIP
9		Proteins	RIA	RIA	RIA	RIA
10		Glycosides	RIA	RIP	RIP	RIP
1		Alkaloids	RIP	RIP	RIP	RIP
2		Carbohydrates	RIA	RIA	RIP	RIP
3		Flavonoids	RIA	RIP	RIP	RIP
4		Triterpenoids	RIP	RIP	RIP	RIP
5		Saponins	RIP	RIP	RIP	RIP
6		Steroid	RIP	RIA	RIA	RIA
7	*Ashwagandha*	Tannins	RIA	RIA	RIP	RIP
8		Starch	RIA	RIA	RIP	RIP
9		Proteins	RIA	RIA	RIA	RIA
10		Glycosides	RIA	RIP	RIP	RIP

Note: Result Indicates Presence (RIP) indicates the presence of secondary metabolites, whereas Results Indicates Absence (RIA) means secondary metabolites is not found in the plant drug.

Fluorescence Analysis of Powdered Drugs with Various Drug Extracts: In order to accurately identify the behaviour of the powdered medicine with different reagents and expose the particular diagnostic properties of the drug to help determine its purity and adulteration, this parameter was utilised. The findings of the current study's testing of powdered leaves of polyherbal (all four herbs) using various test reagents are shown in **Table 5.**

TABLE 5: RESULTS OF FLUORESCENCE TESTS OF ALL FOUR DRUGS, POLYHERBAL POWDER OF LEAVES

S. no.	DP with testing reagent	Vis Radiation Colour	U.V Radiation at 254 nm Colour	U.V radiation at 365nm Colour
1.	DP + NaOH	R	R	DB
2.	DP+ NH_3	Br	Bl	Y
3.	DP + $C_6H_3N_3O_7$	Y	Y B	D B
4.	DP + Con HCl	Sl B	Y B	I B
5.	DP + H_2SO_4	B B	I B	IB
6.	DP + H_2O	C	Y	GY
7.	DP + CH_3OH	Y	Bl	Br
8.	DP +HNO_3	BY	GY	I B
9.	DP + $CHCl_3$	Y	Y	RB
10.	DP + C_6H_{14}	Y	Y	Y

Notions: R; Red, DB; Dark Brown, Br; Brown, Bl; Blue, YB; Yellowish brown, IB; Intense Brown, Sl B; Slight Brown, C; Cream, GY; Greyish Brown, Y; Yellow, RB; Reddish Brown.

Extraction of Plant Material for Biological Activity: To obtain the crude extract, 150 grams of air-dried and finely powdered leaves of all four drugs Plant material was jointly extracted to generate polyherbal extract using ethanol, eight hourin the Soxhlet device. The extract Colour was found Dark brown Percentage (W/W) of obtained 7.34 %.

Biological Evaluation of Polyherbal Extract Obtained From All Four Drug Powder:

Anticancer Potential of Polyherbal Extract (Hepatocellular Carcinoma):

Materials: In addition to MTT solution and kit, other ingredients include Formazan crystals, buffer solution, 96-well plates, micropipets, culture medium, and vortex shakers.

RESULT AND DISCUSSION: We performed *in-vitro* functional assays to more thoroughly explain the anticancer properties of Polyherbal extract using human hepatocarcinoma cancer cells as a model system.

The growth of tumour cell Huh7 cancer cells was shown to be inhibited in a concentration in the **Fig. 1A** & time-dependent manner in **Fig. 1B.** The MTT test was used to evaluate proliferation & survival.

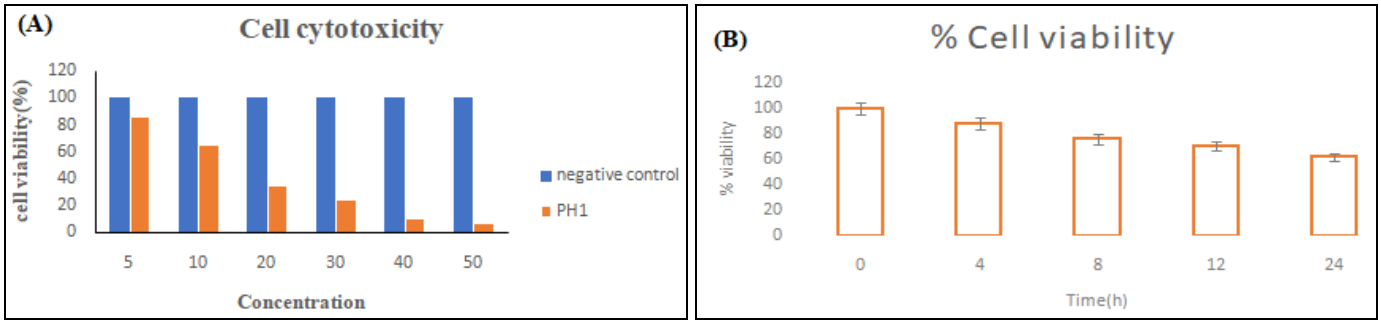

FIG. 1: THE GROWTH OF TUMOUR CELL HUH7 CANCER CELLS WAS SHOWN TO BE INHIBITED IN (A) CONCENTRATION & (B). TIME-DEPENDENT MANNER

CONCLUSION: From the present research work, it is concluded that we can develop a certain potent formulation from known anticancer drugs, as they have already possess known pharmacological activity so we have to assess the quality parameter for the used drugs as in this study, all the standardization parameter has been performed to claim the aforesaid purity and efficacy of the known drugs. Thus in the current study it was found that all the values of quality

parameters were under the limits and allowed the researcher to evaluate the crude extract for the anticancer activity for the well-being of the humans. Hence all results were found very significant to carry out further research and very feasible to go for *in-vivo* studies and also very useful to develop the formulation by using current drugs.

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