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Exploring the Therapeutic Potential of Bottle Gourd-Bael-Triphala Churna in Cancer Treatment

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Abstract: *This study aims to investigate the anticancer activity of the herbal formulation using a hepatocellular carcinoma (HepG2) cell as an in vitro model system. Bael leaves (Aegle marmelos) In the same study, bael may be related to antioxidant and anti-inflammatory effects that protect cells. It is an ancient Ayurvedic formulation of three fruits (Amalaki, Bibhitaki, and Haritaki), known to promote detoxification and maintain gut integrity. Bottle gourd (Lagenaria siceraria) is hydrating, which could enhance the defenses of the body due to its nutrient profile.*
Each of these ingredients is believed to have synergistic benefits for cancer prevention and fighting ability, infectious disease immunity, and maintenance of overall health. In this context, this combination could represent a complementary option of therapy to standard cancer treatments and should be tested in preclinical in vitro and in vivo experiments and subsequently by clinical trials for the evaluation of efficacy and safety in oropharyngeal cancer.

Keywords: Bael leaves, triphala, bottle gourd, anti-cancer activity, herbal formulation, cellular protection, immunity

I. INTRODUCTION

According to the Ayurvedic medical system, churna is a finely ground powder of a medicament or substance. The medications included in Patha are completely cleaned, dried, ground up, and sieved. If kept in an airtight container, the churn will remain potent for a year and be free-flowing. Examples are Drakeshadic hurna, Sudharsana churna, Triphala churna, and Trikatu churna. In the allopathic medical system, churna formulations are comparable to powder formulations. These days, churna is made as pills so that the dosage can be readily adjusted. Because of their particle size, these medications are typically recommended. The absorption rate from g.i.t. increases with decreasing particle size, which in turn increases bioavailability.

Cancer is a general term for the disease that arises when biological alterations lead to unchecked cell growth and division.[1, 2, 3, 4] To put it simply, cancer is a collection of over 100 illnesses that affect uncontrollably growing body cells. Any body tissue can acquire cancer, and each variety of the disease has its own distinct characteristics. Cancer starts when a cell escapes the usual controls on cell division and starts to proliferate on its own. [1, 2, 5] An observation made by "Hippocrates" about 2,300 years ago is where the name "cancer" originates. The Greek word "korkinoma" was followed by the Latin word "cancer." The discovery that all cells are direct progeny of other cells and that living tissues are made up of cells was made by Hooke in the 1600s and Virchow in the 1800s. Death may ensue if the metastasis—the term for the spread of cancer cells—is not stopped. Both internal (inherited mutations, hormones, immunological conditions, and random mutations) and external (tobacco, chemicals, radiation, and infectious organisms) factors contribute to cancer. There are many different, intricate, and still poorly understood causes of cancer. A lot of things are complicated and not fully understood. Numerous risks include environmental pollution, obesity, inactivity, and certain illnesses [6].

Metastasis: Itis the movement or spreading of cancer cells from one tissue to another. Cancer cells usually spread through the blood or the lymph system.

Types of cancer: There are multiple types of cancers today but, some of the common types of cancer are: [5,6,7,8,9]

- Bladder cancer

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- Thyroid cancer
- Breast cancer
- Prostate cancer
- Colon and Rectal cancer
- Pancreatic cancer
- Endometrial cancer
- Kidney cancer
- Lung cancer
- Liver cancer
- Skincancer

1. Bladder cancer : One prevalent kind of cancer that starts in the bladder's cells is bladder cancer. Urine is stored in the hollow, muscular bladder located in the lower abdomen.
   The cells that line the lining of your bladder, known as urothelial cells, are where bladder cancer usually starts. Your kidneys and the ureters, which are tubes that link the kidneys to the bladder, also contain urothelial cells. Although it can occur in the kidneys and ureters as well, bladder urothelial carcinoma is far more prevalent.
   The majority of bladder cancers are discovered early on, while they are still very curable. However, even bladder tumors in their early stages might recur following effective treatment. In order to check for bladder cancer recurrence, patients with bladder cancer usually require follow-up testing for year.
2. Thyroid cancer : Cell growth that originates in the thyroid is known as thyroid cancer. The thyroid is a butterfly-shaped gland situated directly beneath the Adam's apple at the base of the neck. Hormones that control blood pressure, body temperature, weight, and heart rate are produced by the thyroid. At first, thyroid cancer may not show any signs. However, if it worsens, you may have symptoms including neck swelling, changes in your voice, and trouble swallowing.
3. Breast cancer : One type of cancer that starts as a proliferation of cells in the breast tissue is called breast cancer. In the US, breast cancer is the most prevalent cancer diagnosed in women, second only to skin cancer. However, breast cancer is not limited to women. Breast cancer can affect anyone because everyone has some breast tissue from birth.The survival rate for breast cancer has been rising. Additionally, fewer people are losing their lives to breast cancer. This is largely because breast cancer awareness and research funding are widely supported.
4. Prostate gland :In men and those who were assigned male at birth (AMAB), the prostate is a gland located in front of the rectum and beneath the bladder. It is made up of glandular and connective tissues. Its muscles aid in pushing semen through your urethra and give it more fluid. Prostate diseases include benign prostatic hyperplasia, prostatitis, and malignancy.
5. Colon cancer :A cell growth known as colon cancer starts in the colon, a section of the large intestine. The colon is the first and longest part of the large intestine. The large intestine is the last part of the digestive system. The digestive system breaks down food for the body to use.
6. Rectal cancer : One type of cancer that begins as a proliferation of cells in the rectum is called rectal cancer. The final few inches of the large intestine are called the rectum. It begins at the end of the colon's last section and terminates when it enters the anus, a brief, constricted channel.
7. Pancreatic cancer : One kind of cancer that starts as an expansion of cells in the pancreas is called pancreatic cancer. Behind the lower portion of the stomach is the pancreas. It produces hormones that aid in blood sugar regulation and enzymes that aid in food digestion.Pancreatic ductal adenocarcinoma is the most prevalent kind of pancreatic cancer. The cells lining the ducts that transport digestive enzymes from the pancreas are where this type starts.Rarely is pancreatic cancer discovered in its earliest stages, when there is the best possibility of recovery. This is due to the fact that it frequently doesn't show symptoms until other organs have been affected.
8. Endometrial cancer : One kind of cancer that starts as uterine cell proliferation is endometrial cancer. The pear-shaped, hollow pelvic organ where fetal development takes place is called the uterus.

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The endometrium, the layer of cells that makes up the uterine lining, is where endometrial cancer starts. Uterine cancer is another name for endometrial cancer. Although they are far less prevalent than endometrial cancer, other cancers, such as uterine sarcoma, can develop in the uterus. Because endometrial cancer exhibits symptoms, it is frequently detected early. Unusual vaginal bleeding is frequently the initial sign. The uterus can frequently be surgically removed to cure endometrial cancer if it is discovered early.

9. Kidney cancer : Cell growth that begins in the kidneys is known as kidney cancer. Each kidney is roughly the size of a fist and has a bean-like shape. One kidney is on each side of the spine, andThey are situated behind the abdominal organs. Renal cell carcinoma is the most prevalent form of kidney cancer in adults. There are other, less frequent forms of kidney cancer. Wilms tumors, a kind of kidney cancer, are more common in young children.It appears that more kidney malignancies are being diagnosed each year. The increased usage of imaging methods like CT scans could be one factor contributing to this. Additional kidney tumors may be unintentionally found as a result of these examinations. When kidney cancer is tiny and limited to the kidney, it is frequently discovered.
10. Lung cancer : One type of cancer that begins as lung cell development is lung cancer. The twoSpongy organs in the chest that regulate breathing are called lungs. Globally, lung cancer is the primary cause of cancer-related deaths.
    The risk of lung cancer is highest among smokers. The longer and more cigarettes smoked, the higher the risk of lung cancer. The risk of lung cancer is greatly reduced by quitting smoking, even if you have smoked for a long time. Even those who have never smoked can develop lung cancer.
11. Liver cancer: Cancer that starts in your liver's cells is known as liver cancer. The liver is a football-sized organ located above your stomach and below your diaphragm in the upper right section of your belly.The liver can develop a variety of cancers. Hepatocellular carcinoma, the most prevalent kind of liver cancer, starts in the primary hepatocyte. Hepatoblastoma and intrahepatic Cholangiocarcinomasare two considerably less prevalent forms of liver cancer.Liver cancer is a type of cancer that begins in the cells of your liver. The liver is a football-sized organ situated in the upper right portion of your abdomen, above your stomach and beneath your diaphragm. Numerous malignancies can arise in the liver. The primary hepatocyte is where hepatocellular carcinoma, the most common kind of liver cancer, begins. Two much less common types of liver cancer include hepatoblastoma and intrahepatic cholangiocarcinomasare
12. Skin cancer : Skin exposed to the sun is the most common site for skin cancer, which is caused by abnormal skin cell proliferation. However, this prevalent type of cancer can also develop on Parts of your skin that aren't typically exposed to sunlight.Melanoma, squamous cell carcinoma, and basal cell carcinoma are the three main forms of skin cancer. By reducing or avoiding ultraviolet (UV) radiation exposure, you can lower your risk of developing skin cancer. Early detection of skin cancer can be achieved by looking for unusual changes on your skin. Your chances of receiving a successful skin cancer therapy are highest when the disease is discovered early. Parts of your skin that aren't typically exposed to sunlight.

Categories of Cancer

There are five broad categories that indicate the tissue and blood classifications of cancer. [1, 5, 3, 8, 9]

1. Carcinoma: It is the cancer that is found in the tissue known as "epithelial tissue that covers surfaces of organs, glands, or body structures, and there are four main types of carcinomas: they are melanoma, basal cell carcinoma, squamous cell skin cancer, and Merkel cell carcinoma.
2. Sarcoma: It is a malignant tumor growing from connective tissues, such as cartilage, fat, muscles, tendons, and bones. The most common sarcoma is of bone; examples are osteosarcoma (occurs in bone) and chondrosarcoma (occurs in cartilage). There are four types of sarcomas: soft tissue sarcoma, osteosarcoma, chondrosarcoma, and Ewing's sarcoma.
3. Lymphoma: The cancer that originates in the nodes or glands in the lymphatic system; there are three types of lymphoma: Hodgkin's lymphoma, non-Hodgkin's lymphoma, and cutaneous lymphoma.

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4. Leukemia: Also called "blood “cancer” or cancer of the bone marrow, it keeps the bone marrow from producing normal red and white blood cells and platelets. Types of leukemia include acute lymphocytic leukemia, acute myeloid leukemia, agnogenic myeloid leukemia, chronic myeloid leukemia, essential thrombocythemia (ET), hairy cell leukemia, and myelodysplastic syndromes (MDS).
5. Myeloma: It grows in the plasma cells of bone marrow; in some cases, the myeloma cells collect in one bone and form a single tumor that is called a plasmacytoma. However, in some other cases, the myeloma cells collect in many bones, forming many bone tumors; that is called multiple myeloma.

Drug profile:
Bottle Gourd :

- **Synonyms :** lauki, dudhi
- **Kingdom :** plantae
- **Family : curcurbitacea**
- **Orgin :** Africa
- **Genus :** lagenaria
- **Division :** Magnoliophyta
- **Class :** Magnoliopsida

Biological source: Lagenaria siceraria), running or climbing vine of the gourd family (Cucurbitaceae), native to tropical Africa but cultivated in warm climates around the world for its ornamental and useful hard-shelled fruits.
Chemical constituent: Cucurbitacin B, oleic acid, palmitic acid, lagenin, aerpene byonolic acid, olenic acid, omega-3, ferrulic acid, polar extract, sterols, carotenoids, and flavone-C glycosides.
Geographical distribution: Prehistoric distribution and dispersal of the bottle gourd (Lagenaria siceraria) in Asia, the Americas, and Oceania. The bottle gourd has been present in the Americas and East Asia since 10,000 and 7,000 years B.P., respectively (Chang 1986; Smith 2005). In the case of the East Asian bottle gourd, it is unclear how far south it spread in prehistory (indicated by a dashed line). The Southeast Asian bottle gourd may in fact be a much more recent arrival from India, 200 B.C. (Green 2000), and spread only as far east as Vanuatu in prehistory (Yen 1973). The bottle gourd was apparently not present in Western Polynesia (Whistler 1990) (the Bottle Gourd Gap), suggesting that it was not introduced from Asia into Polynesia via human-mediated dispersal (although natural dispersal is still possible).

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However, the bottle gourd may not have been required in the Gap region, as Lapita pottery was widely available as an alternative for containers (distribution of Lapita sites from Kirch [2000]). The bottle gourd was also present in Eastern Polynesia since before A.D. 1,200 (Green 2000) and may have been introduced from the Americas by either natural (floating) or human-mediated dispersal.

Mechanism of action: Cancer is one of the leading causes of mortality worldwide. Many of the cucurbitaceae plants possess antitumor activity. On the basis of traditional use, the present study was carried out to evaluate the anti-cancer activity of methanol extract of Lagenaria siceraria (Mol.) Standley [Cucurbitaceae] aerial parts (MELS) on Ehrlich's Ascites Carcinoma (EAC) model in mice. After inoculation of EAC cells into mice, treatment with MELS (200 and 400 mg kg^{-1}) and the standard drug, 5-Fluorouracil (20 mg kg^{-1}), was continued for 9 days. Evaluation of the effect of drug response was made by the study of tumor growth response, including an increase in life span, study of hematological parameters, biochemical estimations, and antioxidant assay of liver tissue. Experimental results revealed that L. siceraria possesses significant anticancer activity, which may be due to its cytotoxicity and antioxidant properties. Further research is ongoing to find out the bioactive principle(s) of MELS for its anticancer activity. [12] Anticancer activity of methanol extract of Lagenaria siceraria aerial parts has also been reported on Ehrlich's Ascites Carcinoma (EAC) model in mice. After inoculation of EAC cells into mice, treatment with MELS (200 mg and 400 mg/kg) and the standard drug 5-fluorouracil (20 mg/kg) was continued for 9 days. Evaluation of the effect of drug response was made by the study of tumor growth response, including increasing in life span, study of hematological parameters, biochemical estimation, and antioxidant assay of liver tissue. Experimental results revealed that L. siceraria possesses significant anticancer activity, which may be due to its cytotoxicity and antioxidant properties. [13]

Uses:

- Weight loss management
- Reducing the risk of cancer
- Stomach Soothing
- Blood Sugar Regulation
- Helps in cough relief

Properties of bottle gourd:

Anti-hyperlipidemic property: Four separate extracts—chloroform, petroleum ether, aqueous, and alcoholic extracts—were given orally to rats with triton-induced hyperlipidemia in order to examine the antihyperlipidemic efficacy of Lagenaria sciceria. At 400 and 200 mg/kg, p.o., alcoholic and chloroform extracts significantly reduced low-density lipoprotein, total cholesterol, and triglycerides while raising HDL levels. Bottle gourd constituents were discovered to have antihyperlipidemic properties against Triton-X-induced hyperlipidemia. (Ghule and others, 2006) [14]

Antioxidant activity: Using DPPH to extract the acetone from bottle gourd fruit epicarps demonstrated the highest antioxidant capacity against in vitro models. According to Deshpande et al. (2007), the fresh juice exhibits free radical scavenging action. discovered to be highly effective as an immunomodulatory, hepatoprotective, antioxidant, antihyperglycemic, and antihyperlipidemic drug [15]. The extracts were evaluated using the serial extraction method and Soxhlet apparatus in the 1,1-diphenyl 1-2 picrylhydrazyl test. When tested on rats with T4-induced hyperthyroidism, fruit peel extracts at safe dosages of 50, 100, and 200 mg/kg were found to be the most efficacious and could reduce hepatic LPO and blood thyroxine and glucose levels. Following the 21-day course of treatment, decreases in blood thyroid hormone, hepatic LPO, and glucose concentrations were noted. (Dixit and others, 2008) [16]

Diuretic property: By analyzing various parameters, such as total urine volume and urine concentrations of sodium, potassium, and chloride, the diuretic efficacy of Lagenaria siceraria fruit was evaluated. Fruit extracts from Lagenaria siceraria (100–200 mg kg-1, p.o.) were observed to enhance urine volume and electrolyte output in a dose-dependent manner (Ghule et al., 2007).

Anticancer activity : Cucurbitaceae plant family possess antitumor activity. The study was carried out to evaluate the anti-cancer activity of methanolic extracts of aerial parts of Lagenaria siceraria on mice by inoculation of EAC

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(Ehrlich's Ascites Carcinoma) with MELS extracts at 200 mg and 400 mg/kg with the standard drug 5-fluorouracil (20 mg/kg) and continued for 9 days. The effect of the drug response was made by the study, including biochemical estimation and increasing life span and hematological parameters of liver tissue. Results revealed L. siceraria shows significant anticancer activity due to its antioxidant and cytotoxicity properties. (Vichai and Kirtikara, 2006) [17]

Antimicrobial activity : In traditional medicine, methanolic extracts of Lagenaria siceraria's seeds, leaves, and fleshy fruit are widely used to treat skin conditions. Goji used the agar-well diffusion method to assess Ls's antibacterial properties. The results showed that L. siceraria methanolic extracts exhibit activity against Pseudomonas aeruginosa and Streptococcus pyogenes but not significantly against isolates of S. aureus and Escherichia coli. Therefore, skin conditions can be treated with Lagenaria siceraria's antibacterial action. (Goji and others, 2006) [18]

Cardioprotective activity : In rats, the fruit powder from L. siceraria demonstrated strong cardioprotective benefits. The medication, which was administered intraperitoneally (p.o.) for 18 days at a dose of 200 mg/kg to rats to prevent doxorubicin-induced cardiotoxicity, inhibits the reduction of glutathione and antioxidants. In isolated rats, the powdered and ethanolic extracts of LS demonstrated protection against doxorubicin-induced changes in histological alternation, as well as an increase in contraction force and a decrease in contraction rate.Deshpande et al. (2008) [19] Hassanpour et al. (2008); [20]

Bael leaves:

The administration of the aqueous extract of bottle gourd orally reduced the elevated levels of triglycerides, cholesterol, and low-density lipoprotein while enhancing the high-density fruit lowers the cholesterol level. Saponins in this fruit increase lipoprotein activity and rapidly remove fatty acids in the blood (Aslam & Nija

- **Synonyms:** Bilava leaves, Baelpatra
- **Kingdom :**plantae
- **Family : Rutaceae**
- **Origin :**india
- **Genus :**Aegle
- **Division :**Magnoliophyta

Geographical distribution :like Sri Lanka, Pakistan, southern Nepal, Burma, Bangladesh, Vietnam, Laos, Cambodia, Thailand, the northern Malay Peninsula, Java, Timor Leste, the Philippines, and Fiji Bael [Aegle marmelos (L.) Correa] is native to India and is widely distributed throughout the country in 8 states such as Uttar Pradesh, Bihar, West Bengal, Rajasthan, Madhya Pradesh, Uttaranchal, Chattisgarh, and Odisha. Globally, it is also cultivated on a minor scale in countries, etc.

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Chemical constituents: Aegelin, Cineol, Lupeol, Citral, Citronellal, Cuminaldehyde, Marmesinin, Marmelosin, and Skimmianine.

Mechanism of action: According to mechanistic research, Bael's radioprotective benefits are multifaceted and stem from its ability to scavenge free radicals, act as an antioxidant, boost glutathione levels, and reduce lipid peroxidation. In the in vitro systems of investigations, the leaf extract is found to be an effective iron chelator and a strong scavenger of reactive oxygen species and reactive nitrogen species.[21, 22, 23, 24] The fruits' methanol and acetone extracts are also shown to be successful in lowering the SOS response in the chromotest that is triggered by hydrogen peroxide and aflatoxin B1, indicating that they have antimutagenic properties and stop mutagenesis, which is the precursor to carcinogenesis. [25] Bael leaf has been shown to boost the activity of antioxidant enzymes (catalase, glutathione peroxidase, and superoxide dismutase) in mice, irradiated mice, and diabetic rats treated with alloxan. [26, 23]. [27] Along with preventing radiation-induced lipid peroxidation in the mice's liver, kidney, gut, and spleen, the leaf and fruit extracts also caused an increase in glutathione levels. [28,29,23]By interacting with macrophages from the systemic immune compartment, specifically the peritoneal cavity, the leaf extract boosted the immunological response. Given that macrophages are the initial line of defense against microbial invasion and neoplastic disorders, the effect that has been seen has been important.[23]

Uses:

- Treatment of jaundice
- Treatment of wounds
- Used as carminative and astringent

Properties of bael leaves:

Antidiabetic Activity: Experiments on animal models have demonstrated that bael extracts have much stronger antidiabetic activity [30]. On the pancreatic tissues of diabetic rats, bael fruit extracts have shown protective properties [31]. Rabbits developed hyperglycemia when given fruit extracts in both alcoholic and aqueous form at a dosage of 500 mg per kilogram of body weight [32, 33]. When bael fruit extracts were given to rats, hyperglycemia also occurred along with an increase in vitamin C content [34]. Rat models were used to further observe the bael aqueous extracts' antihypoglycemic [36] and hyperglycemic [35, 36] properties. Choudhary et al. [37] used aqueous extracts of bael seeds to further establish these antidiabetic and hyperglycemic properties. The hyperglycemic effect of bael leaves has also been demonstrated [38]. Bael leaf extracts can suppress aldose reductase activity, anticataract activity, and free radical scavenging activity, all of which are linked to diabetes, and they can also lower the expression of the Mi receptor gene, according to Gireesh et al. [39].The antihyperglycemic action of bael leaves is attributed to the component Aegeline-2 [40].

Anticancerous Activity: Of all the noninfectious disorders, cancer is the most bothersome. Herb phytochemicals are frequently heralded as a treasure trove for the development of anti-cancer medications. Numerous studies have been published on bael's anticancer properties. Significant harmful effects were found when Bael's anticancer potential was tested using tumor cells, the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay, hemolysis, brine shrimp lethality, and sea urchin eggs [41]. The body cavity-based lymphoma cell line (BC) (MCF7), mast cell line (MC) (Colo38), and Ehrlich ascites carcinoma (EAC) (LK562) were among the cancer cell lines in which the chemicals extracted from bael demonstrated in vitro anticancer activity [42, 43, 44]. Gas chromatography mass spectrometry (GC-MS) subsequently identified the bioactive compounds found in the bael extracts as 5-fluorouracil, butyl-p-tolyl sulfide, 5-methoxypsolaren, cisplatin, chromomycin, and cytosine arabinoside [44]. Bael extracts, like many other bioactive compounds, have the ability to reduce inflammation and angiogenesis, boost immunity, and modify reactive oxygen species. Bael's anti-inflammatory and antiangiogenesis properties were demonstrated using high-tech, cutting-edge imaging methods such as nuclear magnetic resonance (NMR), positron emission tomography (PET), and single photon emission computed tomography (SPECT) [45]. Chemotherapy and radiotherapy are often employed methods to gradually destroy malignant cells. Determining the appropriate dosages of chemotherapy and radiation treatments to reduce the cytological and genetic harm to healthy cells is a major challenge for oncologists. Because each patient reacts differently to these treatments, it is fairly complex. It has been discovered that certain herbs, like bael, have

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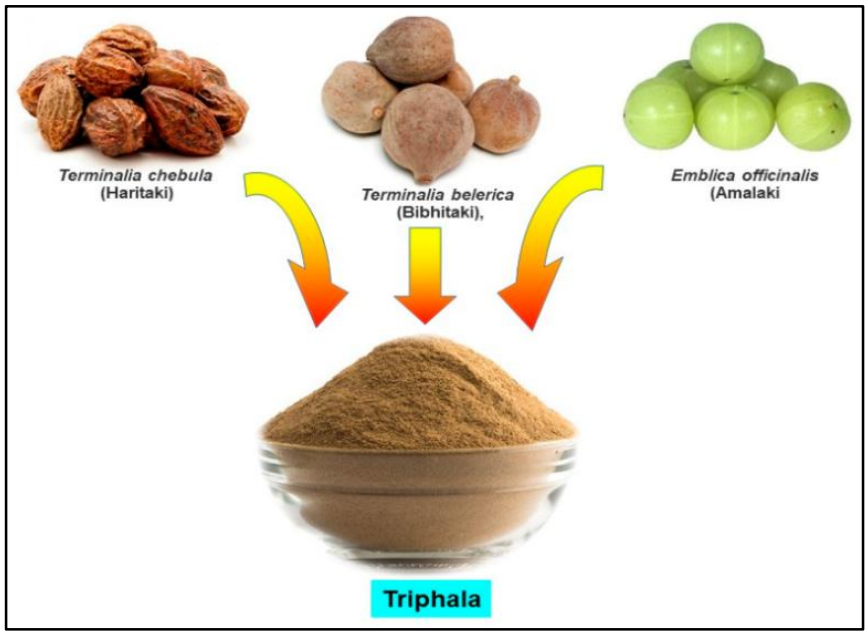
preventive properties against radiation and chemotherapy. Chemotherapy commonly uses the powerful medication doxorubicin, which can also permanently destroy healthy cells' DNA. Nonetheless, it was discovered that bael fruit extract prevented the genetic harm brought on by doxorubicin [46].

Antimicrobial Activity: Extracts from bael have demonstrated antiviral [49, 50], antifungal [48], and antibacterial [47] properties. Bael was shown to have an antibacterial impact on pathogenic Shigella dysenteriae, and the extract's coumarin components were thought to be responsible for the inhibitory activity [51]. The same or related chemicals may also be responsible for the antidiarrheal activity observed in Shoba and Omas [51]. Combining bael with the well-known antibiotic β-lactam as an alternative to traditional antibiotics produced inhibitory activity against S. flexneri and S. dysenteriae that were resistant to β-lactam antibiotics. The differential expression of membrane porins, outer membrane proteins (Omp) C and OmpF, and an Advances in Agriculture study were linked to vulnerability.

Triphala:



Triphala Known for its many health advantages, triphala is one of the most popular herbal compositions in Ayurvedic therapy. "Triphala" literally translates to "three fruits," since it is a concoction of three dried fruits, each of which has special therapeutic qualities. This herbal remedy is highly regarded for its capacity to cleanse, revitalize, and harmonize the body's systems, especially the digestive tract.

Synonyms of Triphala: Vara, Phalatrikam, Sresthatamam, Aksha, Kaliphala, Bhutavasa, Kalidruma, and Karnaphala.

Biological Source: Triphala is well recognised and revered polyherbal medicine consisting of dried fruits of the three plant species Emblica offcinalis,terminalia bellerica, Terminalia chebula. Triphla is a polyherbal formulation made from the dried fruit of three plants, each with its unique properties:

Amalaki (Indian Gooseberry) – *Emblica officinalis* (syn. *Phyllanthus emblica*)
Family: **Phyllanthaceae**
Parts Used: Fruit
Active Compounds: Vitamin C, tannins, flavonoids, polyphenols
Properties: Antioxidant, anti-inflammatory, immune-boosting, anti-aging, digestive aid.
Bibhitaki (Terminalia Bellirica) – *Terminalia bellirica*
Family: **Combretaceae**
Parts Used: Fruit
Active Compounds: Tannins, saponins, flavonoids, ellagic acid

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Properties: Antimicrobial, anti-inflammatory, astringent, digestive stimulant, respiratory tonic.
Haritaki (Chebulic Myrobalan) – *Terminalia chebula*
Family: **Combretaceae**
Parts Used: Fruit
Active Compounds: Tannins, anthraquinones, flavonoids, gallic acid, ellagic acid
Properties: Laxative, antioxidant, anti-inflammatory, antimicrobial, digestive tonic.

Uses:

Triphala is used for gastric disorders such as digestion problems, poor food assimilation, cleansing of colon, constipation and tonifier of the gastrointestinal tract and colon.

It is also recommended to be used for cardiovascular disorders, high blood pressure, serum cholesterol reduction, ophthalmic problems, liver dysfunction, inflammation and complications of the large intestine.(10-11)

It is also used as a blood purifier, to improve the mental faculties and is reported to posses anti-inflammatory, analgesic, ant-arthritic, hypoglycemic and anti-aging properties.

Properties of triphala:

Anticancer activity : Triphala has the ability to kill cancer cell lines. Gallic acid, one of its main components, may be the cause of the inhibition of cancer cell proliferation [52]. Without influencing the healthy breast epithelial cells, it was found that a higher concentration of triphala significantly reduced the viability of breast cancer cells (MCF-7). In MCF-7 cells, triphala increased the intracellular reactive oxygen species (ROS). Triphala caused tumor cells to become cytotoxic but not healthy cells [53]. In Salmonella typhimurium, triphala exhibits antimutagenic activity against 4-nitro-o-phenylene diamine (NDP), sodium azide, and 2-aminofluorene (2AF) in the Ames histidine reversion assay. While the aqueous extract was found to be ineffective, the chloroform and acetone extracts demonstrated suppression of mutationality. Acetone extract was found to prevent revertants caused by S9-dependent mutagens by 98.7% [54]. Two human breast cancer cell lines, MCF-7 and T47D, which differ in their p53 status, show anticancer activity when exposed to triphala [55]. • Compared to T47D, which is p53 negative, MCF7, which has wild-type p53, was more sensitive to triphala. In MCF7 and T47D cells, the exogenous administration of antioxidants glutathione (GSH) and N-acetyl cysteine decreased triphala's antiproliferative properties. In both cell lines, triphala caused an increase in intracellular reactive oxygen species that was dosage and time dependent. The sensitivity of cells to triphala is determined by their P53 state. In both cellular and in vivo models, triphala suppresses the development of human pancreatic cancer cells. Capan-2 cells' ability to survive was greatly diminished after a 24-hour exposure to triphala. When taken orally at 50 mg/kg or 100 mg/kg, triphala inhibited the growth of Capan-2 pancreatic tumor xenografts [56]. Dimethylhydrazine 1,2-dihydrochloride is a very harmful and carcinogenic substance that damages the liver and other important organs. By lowering the lipid peroxidation activity of lactate dehydrogenase (LDH), raising the level of reduced glutathione (GSH), and minimizing peroxidative damage, triphala has a chemoprotective effect against cancer caused by 1,2-dimethylhydrazine dihydrochloride [57]. Triphala can lower the occurrence of tumors by improving the antioxidant status of animals.Triphala lowers the amount of benzo(a)pyrene [B(a)P] that mice are given to treat stomach papillomatosis. In short-term therapy groups, it lowers tumor incidence by 77.77%, whereas in long-term treatment groups, it decreases tumor incidence by 66.66%. [57] When given intraperitoneally to Y-radiation-exposed mice, triphala has a radioprotective effect, delaying the onset of mortality and reducing the symptoms of radiation sickness. Triphala is non-toxic up to a dosage of 240 mg/kg and shows protection at 12.5 mg/kg. When given at a dose of 1 g/kg body weight for seven days before the full-body Y-ray at 7.5 and then for seven days after the radiation, triphala lowers mortality by 60%. • Mice exposed to whole-body Y-rays showed decreased superoxide dismutase activity and increased xanthine oxidase reductase activity in their intestines [58].

Antidiabetic activity : Within four hours, the oral treatment of 100 mg/kg of triphala extract dramatically lowered the blood sugar levels of both normal and alloxan-diabetic rats. The drug's anti-diabetic effects persisted after daily administration. Several studies have examined the potential anti-diabetic effects of triphala in animal models, including diabetic rats generated by a high fructose diet and rats with alloxan [59, 60]. These trials' findings demonstrate that

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administering the extracts lowered blood sugar levels. In vitro, they were shown to scavenge superoxide and hydroxyl radicals and to prevent the production of lipid peroxide [59].

Anti-inflammatory activity : When applied topically, triphala inhibits uveitis brought on by an intravitreal injection of E. coli lipopolysaccharide. Compared to groups that received triphala treatment, the control groups' anterior region inflammation was noticeably higher. Triphala has a preventive effect against uveitis caused by endotoxins. Gallic acid is a specific inhibitor of COX-2, according to one study. Gallic acid is a tiny natural product that selectively and reversibly inhibits COX-2, making it a promising lead molecule for the development of a powerful anti-inflammatory medication [61].

Anti obesity activities : When compared to the control group, mice in an anti-obesity trial that assessed the herbal preparation triphala had lower body weights [62]. Because it is readily available and has anti-obesity properties, gallic acid, a phenolic molecule found in triphala, is chosen as a bioactive marker [63]. In order to assess the effectiveness of triphala in the treatment of obesity, a clinical safety and efficacy trial that is randomized, double-blind, and placebo-controlled is being carried out at Shahed University in partnership with the Endocrinology and Metabolism Research Institute (EMRI) (unpublished data).

Extraction Method:

Extraction method of bottle gourd:

Preparation of sample for dietary fiber (DF) extraction: To remove excess oil, organic acids and salts, and soluble sugars, bottle gourd seed powder (BSP) was processed with 90% ethanol. BSP was blended at 1:10 (w/v) with ethanol, and centrifugation was done at 5000 rpm for 10 min at 20 °C after being constantly agitated at 350 rpm for 15 min with a magnetic stirrer. The residue was kept overnight at 60°C in a hot air oven for proper drying, following which it was stored in an airtight container.

Alkaline extraction: The alkaline extraction method for dietary fiber (AEDF) was slightly modified from the approach described by Zhou et al. (2020). The deoiled bottle gourd seed powder was mixed in NaOH solution of 0.5 M concentration and stirred with a laboratory stirrer at 450 rpm for 30 min at 25 °C. The solution was then neutralized with 0.5 M HCl and centrifuged at 6500 rpm for 15 min at 20 °C. The particulate was recovered and rinsed in 80% ethanol (3:1, w/v) before being centrifuged for another 10 min at 20 °C at 5000 g. The residue was then dried overnight at 50 °C to get AEDF.

Ultrasound-assisted extraction method and its optimization: Ultrasound-assisted alkali extraction of dietary fiber was performed with 0.5 M NaOH using an ultrasonicator with a probe (Q700-220 Digital Sonicator, Qsonica LLC, USA) as followed by Kumari (2022). The parameters for ultrasound-assisted were fixed after performing preliminary experiments. Processing conditions that were considered for extraction included solute:solvent (1:30–1:90), ultrasound amplitude (10–70%) and ultrasound time (10–60 min). Central composition design was used for optimizing the processing conditions for extraction. Extraction was carried out by mixing the sample with alkali and giving ultrasound treatment under controlled conditions. After alkaline digestion, the sample was neutralized with 0.5 M HCl and was centrifuged at 7000 rpm for 30 min at 20 ◦C. Following centrifugation, the residue obtained was washed with 80% ethanol and dried overnight at 50°C in a hot air oven to obtain dietary fiber (UAEDF).

Enzymatic extraction: Dietary fiber was extracted by using enzymes, α-amylase, protease, and amyloglucosidase. Heat-stable α-amylase was used to gelatinize dried powder samples by heating at 95°C for 35 min. Then digestion was performed with protease to remove protein and amyloglucosidase to remove starch. After digestion, the samples were filtered, centrifuged at 7000 rpm for 20 min at 20 ◦C, and then washed with distilled water. Following which, the residues were dried overnight at 55°C in a hot air oven to obtain the dietary fiber (EADF).

Extraction method of Bael leaves:

Aqueous extraction: Bael leaves, after washing, were thoroughly hot air-dried for 3 days at room temperature (~ ~25 ◦ C). The dried leaves were pulverized using a pestle mortar to obtain a powdered form, which was stored in airtight glass vials at 4 ◦C until used. The powdered plant material was mixed with distilled water (1:5) and stirred overnight at room

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temperature. The residue was removed by filtration through Whatman no. 1 filter paper, and the aqueous extracts were lyophilized and stored in airtight glass vials [13]. All extracts were stored in sterilized containers at − −20 °C until used for testing. All extraction and subsequent analyses were done using three replicates.

Alcoholic Extract: The leaves of A. marmelos were collected, shade-dried, and pulverized into coarse powder using a digital grinder. Next, 50 g of A. marmelos leaf powder was accurately weighed and then extracted with 400 mL of ethanol and transferred to a flask in a water bath at 40 °C for 3 h, stirring occasionally. It was filtered through No. 1 Whatman filter paper and then washed with a fresh solvent to remove any remaining residue. The filtrate and washing were pooled and evaporated to dryness on a rotavapor below 60°C. The obtained residue was stored in well-closed containers for further studies. Additionally, the obtained alcoholic extract contained both polar phytoconstituents and nonpolar phytoconstituents.

Extraction method of Triphala:

Aqueous Extract of Triphala: 30 g of Triphala churna (mixture of the dried powder of T*erminalia chebula*, *Terminalia belerica, and Emblica officinalis*, in the ratio of 1:1:1) (IMPCOPS, Chennai, India) was boiled in 100 ml of Milli-Q water till the volume was reduced to 1/3 and was filtered through Whatmann filter paper (Sigma-Aldrich, St. Louis, MO, USA). The filtrate was centrifuged at room temperature to remove debris. The supernatant was lyophilized to get the powdered aqueous extract of triphala (AqE). AqE dissolved in PBS at a concentration of 3 mg/ml was filter sterilized using a 0.22 µm membrane filter (Millipore, Massachusetts, USA) before treating the cells.

Alcoholic Extract of Triphala:50 g of Triphala churna was extracted in 150 ml of ethanol using a Soxhlet apparatus. The extract was evaporated in a vacuum and dried at 40°C to get the alcoholic extract of Triphala (AlE). AlE, dissolved in PBS containing 0.1% DMSO at a concentration of 3 mg/ml, was filter sterilized before treating the cells.

Evaluation of physical parameters:

Determination of pH: With the use of an Elico pH meter, the pH of a 1% solution of the churna formulation was measured.

Calculating the Moisture Content: Mettler Toledo halogen moisture determination equipment was used to determine the moisture content of the churna.

Calculating Ash Values

Value of All Ash: In a silicon crucible that had been previously burned and tarred, two grams of churna were precisely weighed. After that, the substance was ignited by progressively raising the heat to 500–600°C until it turned white, signifying the lack of carbon. After cooling in a desiccator, the amount of total ash in milligrams per gram of air-dried material is computed.

Value of Acid-Insoluble Ash: After adding 25 milliliters of distilled water and letting it slowly boil for five minutes, roughly 5 milliliters of hot water were added, and the crucible was then filled with total ash. An ashless filter paper was used to gather the insoluble material. After that, the filter paper and the insoluble material were placed in a crucible and burned to a constant weight. The filtrate was then cleaned with hot water until, letting the residue cool, it was weighed. It became neutral. After letting the residue cool, it was weighed.

Determination of Extractive Values:

I. Water Soluble Extractive Value :5 grams of churna were accurately weighed and placed inside a glass-stopper conical flask. It is then macerated with 100 ml of chloroform water for 18 hours. It was then filtered, and about 25 ml of filtrate was transferred into a china dish and evaporated to dryness on a water bath. It was then dried at 105°C for 6 hours, cooled, and finally weighed.

II. Alcohol Soluble Extractive Values: Ethanol was used as a solvent in place of chloroform water, and the remaining procedure was the same as that of water-soluble extractive value.

5) Determination Of Crude Fibre Content:2 grams of accurately weighed churna was placed in a round- bottom flask, and then 100 ml of 0.128 M sulfuric acid was added and refluxed for 1 hour, then filtered through ashless filter paper, and the residue was washed with water until the filtrate became neutral. The residue was then weighed (a),

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assigned to ash, and finally the weight of ash (b) was determined. The difference between a and b represented the crude fiber content and was calculated on a dry weight basis.

Determination of Heavy Metal Contamination:

Arsenic Contents: Preparation of Standard Solution (10 PPM) 0.33 grams of arsenic trioxide were dissolved in 5 ml of a 2 M sodium hydroxide solution and then diluted to 250 ml with water. One volume of this was then diluted to 100 volumes with water.

PREPARATION OF SAMPLE

Preparation of Churna solution: The churna solution was prepared by means of diluting 1 gram of churna to 100 ml using distilled water. This is used to carry out limit tests for iron and lead and also to perform qualitative tests for mercury. 10 ml of churna solution was pipetted out into a flask, and about 10 ml of concentrated nitric acid was added and evaporated to dryness on a water bath. The residue was then dried at 130°C for 30 minutes, and then about 10 ml of hydrazine molybdate reagent was added and refluxed for 20 minutes. The solution was then cooled, and the absorbance of both the standard and test solutions was measured at 800 nm using a Perkin Elmer UV spectrophotometer.

Limit test for Iron: Preparation of Standard Solution (20 PPM) One volume of 0.1726% w/v solution of ferric ammonium sulfate solution was diluted in 0.05 M sulfuric acid to ten volumes using distilled water.

Procedure: A limit test was performed in Nessler's cylinder. 2 ml of test and standard solutions were taken in separate cylinders, and then 2 ml of a 20% solution of citric acid and 0.1 ml of thioglycollic acid were added. The solution was then mixed and made alkaline with iron-free ammonia, diluted to 50 mL with distilled water. It was then allowed to stand for 5 minutes, and the color obtained in the sample was compared with that of standard color. If the color produced in the test is greater when compared to that of the standard solution, then the sample was said to fail the limit test and said to pass the test if vice versa occurs.

Limit Test For Lead: Preparation of Standard (20 PPM) 0.4 gm of lead nitrate was dissolved in water containing 2 ml of nitric acid and sufficient water to produce 250 ml. About 1 volume of the above solution was diluted to 10 volumes using distilled water.

Procedure :A limit test was performed in Nessler's cylinder. 1 ml of standard lead solution and test solution were taken in separate cylinders and were diluted to 25 ml using distilled water. Original Article Sri Ramachandra Journal of Medicine Nov. 2007 N41, and then pH was adjusted to a value 3-4 by adding dilute acetic acid or dilute ammonia solution and then diluted to 35 ml using distilled water. To both solutions, 10 ml of freshly prepared hydrogen sulfide solution was added, mixed, and diluted with water to 50 ml. It was then allowed to stand for 5 minutes and viewed downwards over a white surface. The color produced in the test solution should not be more intense than that of the standard solution; if so, then the sample is said to pass the limit test for lead.

Test for Mercury : To 10 drops of the test solution, 6 M HCl was added to get a white precipitate. The precipitate was then treated with 6 M ammonia solution. If the color of the precipitate changes to a gray or black color, then it indicates the presence of mercury.

DETERMINATION OF MICROBIAL CONTENT

1 gram of churna was dissolved in lactose broth, and the volume was adjusted to 100 ml with the same medium. About 10 ml of sample was transferred into 100 ml of MacConkey broth and incubated for 18-24 hours at 43-45 °C. A subculture was prepared on a plate with MacConkey agar and incubated at 43-45°C for 18-24 hours. The growth of red, generally non-mucoid colonies of gram-negative rods appearing as reddish zones indicates the presence of E. coli; if not, then it indicates the absence of E. coli.

Determination of Digestive Property

Preparation of Extract: About 100 mg of an accurately weighed quantity of churna was extracted with 20% aqueous glycerol and phosphate buffer (pH 7.8) in a 1:4 ratio and filtered, and the filtrate was used as an enzyme source. The standard sample was prepared similarly to the test sample.

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Physical Parameters :

Angle of repose: Weighing the reasonable angle at which the minute particles in the air surface detracted toward the level surface was a prerequisite for estimating the angle of repose. Initially, the 100.00 g granules were loaded and fled somewhat into a channel that was created to go along with a lower tier closure. The lid was previously removed, allowing the granules to fall onto the lowermost portion of the pictorial paper surface. Weighing the altitude (h) and distance (d) of the formed granules allowed us to determine the repose angle (α), and the wealth of liquid balancing was used to carefully consider adding the principles into the final seed's equating intention.

$\theta = \tan\text{-}1(h/r)$

Bulk density:

1. Take the 5gm sample powder of known volume (cm^3).
2. Add into 25 ml Measuring cylinder.
3. Measure the volume of sample in measuring cylinder.
4. Notify the both mass and volume of the powder.
5. Calculate the bulk density.

Bulk density = Mass / Bulk volume × 100

Tapped density: The granules were judged by equating the most and pumped capacities of the fleeing granules as well as the rates when they were full below. The principle got was delimited as the portion of uninterrupted book, as premeditated in this manner:

Tapped Density = Weight taken / Tapped volume

Compressibility / Carrs index :

Carrs index = Tapped density – Bulk density / Tapped density ×100

Hausners ratio :

Hausners Ratio = Tapped density / Bulk density

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