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Review Article

A Review On In-Vitro Evaluation Of Antibacterial And Antioxidant Activity Of Polyherbal Formulation: Triphala Churna

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ABSTRACT

Triphala churna is a polyherbal concoction that has been used for a long time in the traditional Indian medicinal system. It has outstanding therapeutic efficacy and is abundant in antioxidants. Ayurveda states that the raw components for the churna should be prepared in equal parts (1:1:1) utilizing Amalaki (Indian Gooseberry), Haritaki (Indian Hog Plum), and Bibhitaki (Vibheetaki). The main goal of the current study was to assure the antioxidant and antibacterial properties of triphala churna using various bacterial strains. Numerous cross-check investigations have been conducted to evaluate the quality attributes of herbs, including the effectiveness and formulation assurance. These analyses include phytochemical detection, physico-chemical analysis, and pharmaceutical analysis. The antioxidant and bactericidal properties of the aqueous and ethanolic extracts of polyherbal drugs are also compared. Using the FRAP experiment and the 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method, the antioxidant capacity of triphalachurna was ascertained. Utilizing a broth dilution, the antibacterial properties were assessed. The results showed that triphala included significant amounts of bioactive compounds, such as phenols, alkaloids, and flavonoids, which may be in charge of particular metabolic processes. Using extracts, a radical scavenging effect akin to that of ascorbic acid was established. The active ingredient in triphala inhibited both gram-positive and gram-negative bacteria, indicating the extracts' promising antibacterial properties.

INTRODUCTION

Multi-herbal medicine compositions are referred to as polyherbal formulations. The concept of polyherbs has existed for more than a millennium and is primarily found in Ayurveda and other various traditional medicinal systems. The theory involves using numerous herbs in a specific proportion to heal a disease, with the aim of enhancing therapeutic activity and minimizing unpleasant occurrences. Ayurvedic literature such as the "Sharangdhar Samhita" expounded on the notion of "polyherbal formulations or

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polyherbalism," which contributes to improved medicinal efficacy. It is impossible for a single plant's active phytochemical components to produce the required medicinal effect. Numerous illnesses are managed with polyherbal medications, such as diabetes, cardiometabolic issues, renal issues, hypertension, liver, and skin disorders. They are known to have antioxidant qualities and lower concentrations of individual herbs, which lowers the possibility of unfavorable outcomes. Polyherbal medications can serve as a substitute for or supplement to single herbal medicines. What makes them up are, multiple herbs that work together to support health and well-being. When mixed in a specific ratio, many plants have a higher medicinal impact and aid in reducing toxicity. To guarantee that polyherbal formulations are safe, sufficient, effective, and palatable for use, they must be evaluated. Polyherbal expression standardization is essential for evaluating the standards of the drug's active ingredient. Evaluation of its medicinal components, physical, chemical, and phytochemical properties, additionally in-vitro and in-vivo standards, form its basis.

The significance of Herbal Formulas: Both major and mild medical diseases have benefited greatly from the use of polyherbal medicines. The therapeutic potential of polyherbal medications is emphasized in the text that follows.

- Their consumer base is large.
- They provide a more sympathetic experience.
- The quality, potential, and well-being of herbal medications have improved because of the advancement of modern technology and information.
- In contrast to other pharmaceuticals, polyherbals are less expensive.
- They don't seem to have any harmful or hazardous consequences.
- Extended use of PHF could validate its safety and efficacy.
- It is well knowledge that medicinal plants and herbs are a sustainable source of medications.

Triphala Churna: Native to the Indian subcontinent, triphala is a popular and potent polyherbal remedy prepared from the dried fruits of three distinct plant species: Terminalia chebula (Family Euphorbiaceae), Terminalia bellerica (Family Combretaceae), and Phyllanthusemblica (Family Euphorbiaceae). Triphala churna is categorized by Ayurveda as a tridosha or tridoshic rasayana due to its ability to prolong life and invigorate people of all ages. As per "The Ayurvedic Pharmacopoeia of India," the composition has three herbal fruits in dried form: Amalaki, which is Indian gooseberry, Haritaki, which is Indian hog plum, and Bibhitaki, which is Vibheetaki, in equal amounts. Indian medicine has been using it for millennia.Many health advantages of triphalachurna include controlling blood sugar, promoting healthy digestion, assisting with weight loss, reducing inflammation, lowering cholesterol, restoring normal blood pressure, and enhancing circulation. It possesses antibacterial, anti-erythrogenic, and antioxidant qualities. In addition to helping with healthy digestion and absorption of nutrients, triphala churna is also used to cure gout and arthritis, mend skin disorders and treat stomach ulcers. Given the wide range of therapeutic benefits associated with triphala, it has been proposed that we concentrateon phytochemical investigations of this underutilized formulation and examine its potential for use in medicine. Consequently, three distinct components were accustomed to make the triphala formulation, following the extraction of the phytochemicals from the herbal remedy triphala using two distinct solvents—triphala aqueous extract (AET) and ethanol extract (EET)—the plant's antibacterial and antioxidant properties were examined in-vitro.

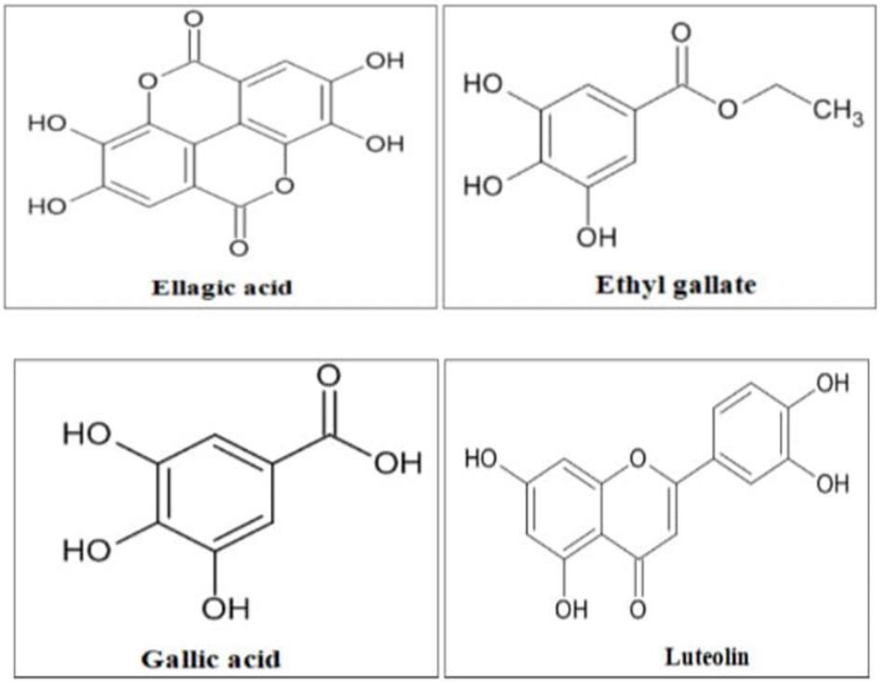

Fig. 1: Compounds of TriphalaChurna known for Antioxidant activity

MATERIALS AND METHODS:

Acquisition of Sample: As illustrated in Fig. 2, the laboratory-prepared triphala components—Amalaki (Indian Gooseberry), Haritaki (Indian Hog Plum), and Bibhitaki (Vibheetaki)—were taken, all of which have been verified as authentic by abotanist. The cost of polyherbal remedies is lower than that of other drugs. They don't seem to have any harmful or poisonous consequences. Extended use of poly herbal formulations could validate its efficacy and safety. Herbal remedies or plants are recognized as a sustainable supply of medication.

Fig. 2: Crude drug specimens of A) Amalaki, B) Haritaki, C) Bibhitaki

Method for Making Triphala Churna: The raw herbs were allowed to dry at ambient temperature in the shade. Next, as demonstrated in Fig. 3, every herb was processed into a fine powder and passed through sieves. This process transformed the raw drugs into an extremely fine powder. It is thereafter meticulously blended in a 1:1:1 ratio in compliance with pharmacopoeial guidelines, and then stored in an airtight container. The

preparation was then put through many quality assurance tests.

Fig. 3: Laboratory formulation of TriphalaChurna

Chemicals and Test Microorganisms: Analytical substances were the only ones used in the experiment. The analytical quality solvents and chemicals needed for the phytochemical analysis, antioxidant, and antibacterial studies were employed in the experiments. Microorganisms include Pseudomonas aeruginosa, Bacillus subtilis, Escherichia coli, and Staphylococcus aureus.

Establishing Standards and Quality Attributes Parameters for Polyherbal Drugs:

Determination of Physico-Chemical Parameters: Physiochemical analysis is a technique for examining a material to ascertain its general purity and stability as well as its physical and chemical properties, i.e., to recognize and measure each component of the material. Extractive values for ethanol, foreign organic matter, total ash, water soluble ash, acid-insoluble ash, and moisture content were evaluated for the sample.

Pharmaceutical Analysis: The study of the chemical and physical characteristics of chemicals derived from plants that are employed in the pharmaceutical industry is known as pharmaceutical analysis. Numerous identification and quantification tests are part of this. The plant material's flowable qualities were themain focusof this particular pharmacological test. The sample's apparent and tapped densities, angle of repose, Carr's compressibility index, and Hausner's ratio were all measured.

Phytochemical Analysis: A variety of potent bioactive molecules or substances that could be the source of their medicinal capabilities can be observed and validated by phytochemical evaluation procedures. Preliminary testing of phytochemicals was completed on the material after the aqueous and ethanolic extracts of the polyherbal medicine were synthesized in a lab. Alkaloids, carbohydrates, tannins, saponins, steroids, protein, flavonoids, phenol, and amino acids are all detected by this test.

Antibacterial Activity Determination: The antibacterial property of the aqueous and ethanolic extract was evaluated against gram-positive bacteria such as Bacillus subtilis and Staphylococcus aureus, as well as gram-negative bacteria like Escherichia coli and Pseudomonas aeruginosa. An isolated colony from agar plates was added to the Luria broth (LB) medium to establish overnight cultures, which were subsequently cultivated for 12 hrs at a temperature of 37° C. The nocturnal cultures were diluted to approx 104 CFUs and then incubated at 37° C for 12–14 hours to assess how the overnight cultures grew in comparison to the control culture, which was limited to media and the bacterial inoculum, both on brand-new LB medium. The study was conducted twice more for confirmation. The

%MGI (Mean Growth Inhibition) is calculated using the following formula, %MGI=dc-dt/dc×100

Antioxidant Activity Determination:

-DPPH Free Radical Scavenging Method: The antioxidant activity of the polyherbal medicine, its constituents, and ascorbic acid (as standard) was evaluated based on the stable DPPH's capacity to scavenge free radicals. The polyherbal medication was made in several diluted aqueous and ethanol solutions. Standardization was done by using ascorbic acid (1 mg/ml) and purified water. Working sample solutions were taken and combined with 500 milliliters of the 0.1 mM DPPH solution. These solutions were kept in a dark area. Using a spectrophotometer, the optical densities of various solution combinations were determined at 517 nm following 30 minutes of darkness. 0.1 mM DPPH was the solution utilized as the control. A specific quantity of diluted ethanolic and aqueous extracts were utilized as a control. After measuring the optical density, the following formula was used to calculate the amount of DPPH free radical scavenging, where the absorbance at 517 nm of the test sample and the control sample are denoted by dc and dt, respectively.

-FRAP Assay: A few modest adjustments were made and developed to quantify antioxidant activity for the ferric reducing antioxidant power (FRAP) assay. Five milliliters of TPTZ solution (10 mM) produced in HCl (40 mM), five milliliters of FeCl3 (20 mM) water solution, and fifty milliliters of acetate buffer (0.3M) at pH 3.6 were combined to create the FRAP reagents. In ethanol and distilled water, various diluted working solutions of Triphala and its constituent plants were created. After each sample (200 ml) was mixed with 1.5 ml of freshly generated FRAP solution for five minutes, absorbance was measured at 587 nm using the FRAP working solution as a reference. Standardization was achieved using ascorbic acid. The results of the ethanolic and aqueous extracts were expressed in mM Fe2+/ml. A larger reducing power is correlated with a higher absorption.

RESULTS AND DISCUSSIONS:

Physico-Chemical Analysis: Positive outcomes were derived from the physico-chemical investigation of the polyherbal medication formulation. The outcomes were within the WHO and Indian Pharmacopoeia's acceptable ranges. Table 1 presents the triphala preparation results.

Table 1: Results for physico chemical evaluation

Attributes (%)	Triphala Preparation	Standard (IP)
Foreign matter	Nil	Less than 3.0%
Moisture content	9.6 ± 0.023	Less than 12.0%
Water soluble extract	4.46 ± 0.785	Less than 35.0%
Alcohol soluble extract	23.2 ± 0.623	Less than 25.0%
Total-ash value	8.2 ± 0.026	Less than 8.0%
Acid-insoluble ash	2.82 ± 0.105	Less than 3.0%

Pharmaceutical analysis: Triphala's pharmacological study showed that the polyherbal medication had poor flowable qualities, reporting a tapped density of 0.539 and an apparent density of 0.391. Carr's index (27.45) and Hausner ratio (1.37) indicate that it has poor to extremely poor flow characteristics and exhibits a passable sign via angle of repose. Table 2 presents the preliminary triphala preparation results.

Table 2: Results for Pharmaceutical analysis

Attributes	Triphala Preparation
Apparent density	0.391 ± 0.12
Tapped density	0.539 ± 0.19
Carr's index	27.45 ± 0.34
Hausner's ratio	1.37 ± 0.25
Angle of repose	44.6 ± 0.13
pH	3.4 ± 0.1

Phytochemical Analysis: The results of the phytochemical evaluation of AET and EET showed the presence of different phytoconstituents and were examined using chromogenic reactions. Major phytoconstituents including tannins, saponins, steroids, phenol, and flavonoids were reported to be present by both AET and EET. Compared to the water extract, the ethanolic extract produced an extraordinary chromogenic reaction, indicating that the ethanol solvent removed more phytoconstituents. Table 3 presents the findings derived from AET and EET.

Table 3: Results for Phytochemical Analysis

Phytoconstituents	Tests	Triphala extracts	
		AET	EET
Alkaloids	Hager's test	-	-
	Mayer's test	-	-
	Wagner's test	-	-
Carbohydrates	Benedict's test	+	++
Tannins	Braymer's test	+	++
Saponins	Foam test	+	+
Steroids	Salkowski's test	+	++
Proteins	Biuret test	- -	
Amino acids	Ninhydrin test	- -	
Phenols	Lead tetra acetic acid test	+	++
Flavonoids	Shinoda test	+	++

"++" indicates the presence of appreciable amount. "+" indicates the presence of moderate amount. "- "indicates the absence of phytoconstituents.

Antibacterial Activity: The % MGI was obtained using the polyherbal medication triphala, and many bacterial strains were utilized to determine the antibacterial properties of the ethanolic (EET) and aqueous (AET) extracts. The outcomes were similar to those of the beta-lactam antibiotic ampicillin, which is a common antibiotic. The medium used for bacterial cultures was all that was required for a positive control. As per the results, triphala exhibited bacteriostatic qualities in vitro, which implies that both the extracts inhibited the growth of microorganisms, as shown in Fig. 4. AET was found to have the most inhibiting action against B. subtilis (95.030 ± 0.411%), as illustrated, whereas it had the lowest inhibitory action against P. aeruginosa (36.446 ± 0.251%). In ethanolic extracts, EET was found to have the greatest inhibitory effect against B. subtilis (82.226 ± 0.396%), while P. aeruginosa showed the least amount of inhibition (52.241 ± 0.411%). For E. Coli, ampicillin had the most effect (MGI% 98.204±0.498), whereas for B. subtilis, it had the least effect (MGI% 88.68 ± 0.478). EET is the most effective antibacterial agent because, as the observations mentioned above demonstrated, it exhibited considerable suppression against all the tested strains of bacteria. In general, the findings indicate that triphala functions as a broad-

spectrum antibacterial agent. According to Fig. 4, triphala exhibits more potential when compared to gram-negative and gram-positive bacteria.

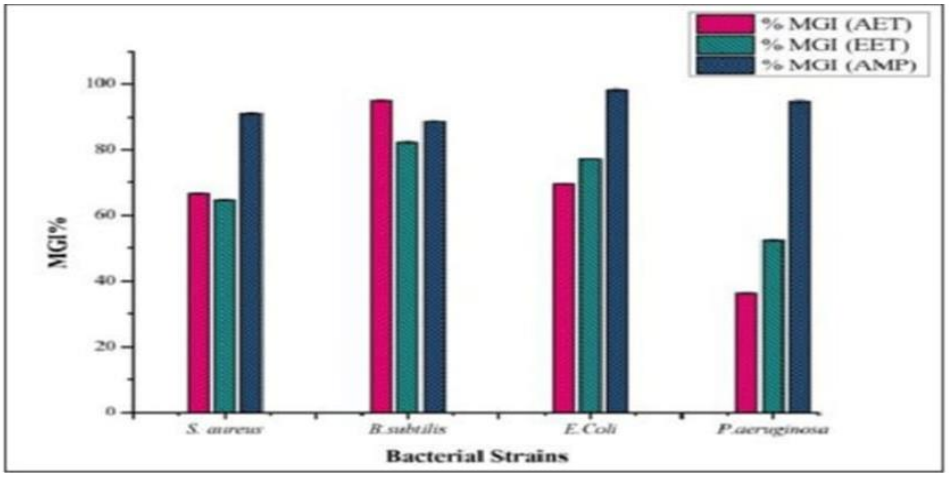

Fig. 4: Depictions of comparison of In-Vitro Antibacterial Activity of both gram-negative and gram-positive bacteria by Broth dilution test represented using Bar diagram. The bars show the %MGI that Triphala Churna extract was found to have when tested using ethanolic and aqueous extracts.

Antioxidant Activity:

-DPPH: The DPPH radical-scavenging abilities of the AET and EET were investigated, and both extracts demonstrated a significant level of DPPH scavenging ability. The scavenging ability of each extract was compared to that of ascorbic acid. Using AET, the antioxidant activity of DPPH was assessed and contrasted with that of ascorbic acid and EET. The extract with the greatest and lowest levels of radical scavenging activity was found in volumes containing 50 µL and 500 µL, respectively. AET showed DPPH free radical scavenging activity at a minimum of 10.48±0.25% and a maximum of 61.94±0.42%. The extent to which EET can scavenge DPPH radicals rose to 96.35 ± 0.37% from 19.18 ± 0.43%. The graph showed that EET has a maximum DPPH activity of 93.39 ± 0.29% and a minimum of 84.35 ± 0.32%, indicating a high degree of free radical scavenging activity in proportion to ascorbic acid (Vitamin C). In terms of DPPH scavenging, the EET closely lagged the AET, as shown in Fig. 5.

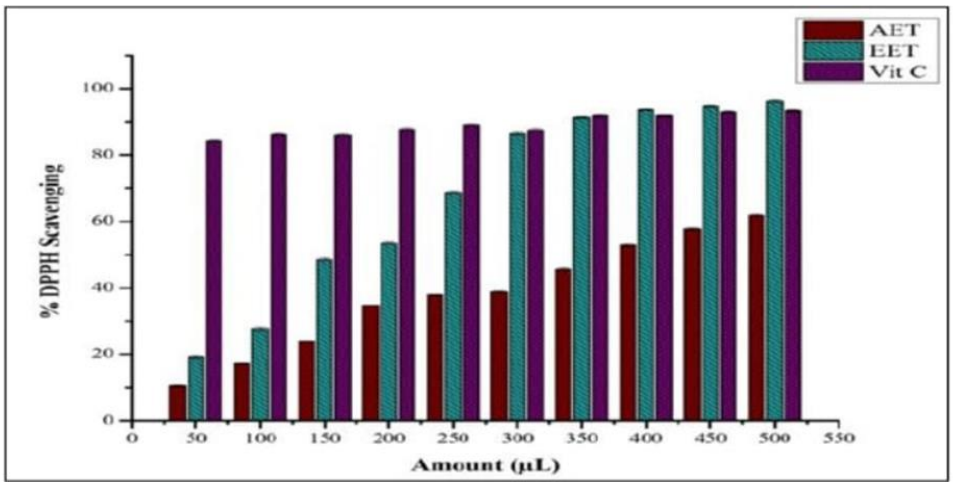

Fig. 4: Depictions of comparison of Antioxidant activity of Ethanolic and Aqueous Extracts of Triphala Churna by DPPH Free Radical Scavenging Method

-FRAP Assay: The FRAP test was used to evaluate how well the plant extracts reduced ferric ions. The Fe (III)-TPTZ combination would be reduced to Fe (II)-TPTZ, which absorbs significantly at 593 nm, by an antioxidant having one electron to contribute. In triphala, it was discovered that the antioxidant effectiveness of FRAP was concentration-dependent. For the FRAP test, both the ethanolic and aqueous extracts were examined. The obtained outcomes were compared to the reference point. The concentration-dependent FRAP impact was observed in 50 µL of ascorbic acid and 500 µL of both extracts. As illustrated in Figure 5, AET and EET revealed maximum and minimum values, respectively, of 1.04 ± 0.018 mM and 0.19 ± 0.003 mM and 1.02 ± 0.016 mM and 0.22± 0.002 mM. Based on observations, triphala has shown satisfactory performance in comparison to ascorbic acid. AET and EET both displayed the greatest FRAP value at 500µL of extract.

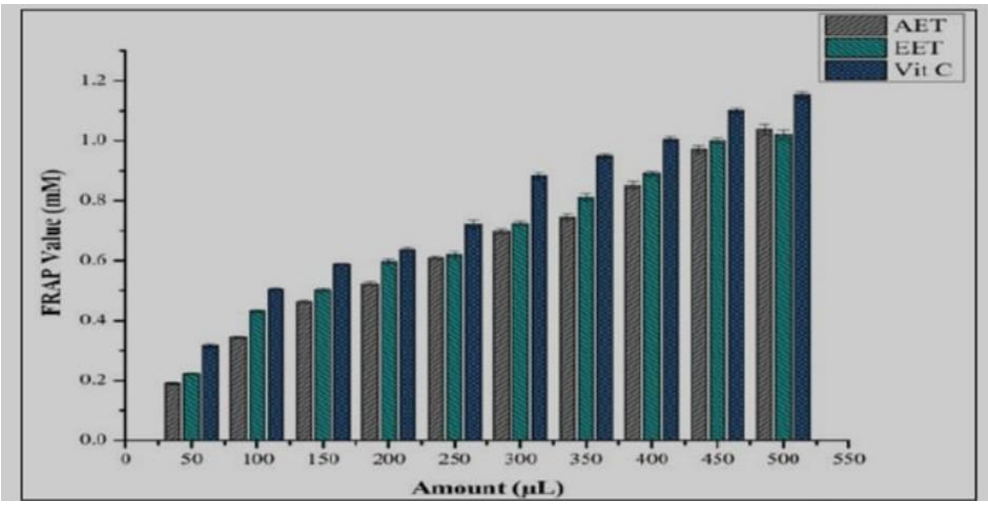

Fig. 5: Depictions of comparison of Antioxidant activity Ethanolic and Aqueous Extracts of Triphala Churna by FRAP Assay Method

CONCLUSION:

In this study numerous standardized features are examined, including comparative analysis, phytochemical analysis, medicinal evaluation, and physico-chemical standards. This study also shows that triphala and its components have demonstrated potent antibacterial and antioxidant qualities. As a result, it might be a good source of natural compounds that are antibacterial and antioxidant. This review leads one to the conclusion that further research should be done on the plant to find novel chemicals obtained from it that may be more effective as antioxidants and antimicrobials. To find and isolate bio-active molecules for a thorough evaluation of their in-vivo action, more study on triphala and its component parts is necessary.

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