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IN-VITRO ANTIBACTERIAL AND ANTIOXIDANT EVALUATION AND QUALITY ASSESSMENT OF POLYHERBAL DRUG: *TRIPHALA CHURNA*

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Keywords:

Polyherbal, Standardization, Antioxidant, Antibacterial, Quality attributes

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ABSTRACT: *Triphala Churna* is a poly-herbal preparation that has anantiquity of usage in the conventional Indian medical system. It is rich in antioxidants and has excellent therapeutic efficacy. According to ayurveda, the churna is made using the raw ingredients Amalaki (Indian Gooseberry), Haritaki (Indian Hog Plum), and Bibhitaki (Vibheetaki) in an equal proportion (1:1:1). The present investigation purpose was to establish the Triphala's antimicrobial effect on different types of bacterial stains and its antioxidant effects. Numerous cross checks examinations have been conducted, such as phytochemical detection, physico-chemical analysis, pharmaceutical analysis, to evaluate herbs' quality attributes, including formulations' assurance and effectiveness. A comparison of the antioxidant and antibacterial capabilities of polyherbal drug's aqueous and ethanolic extract follows. Antioxidant activity of *Triphala churna* was established by FRAP assay and 2,2-diphenyl-1-picrylhydrazyl free radical scavenging methods. By using broth dilution, antibacterial potential was assessed. Triphala reported the presence of valuable bioactive components, including phenols, alkaloids, and flavonoids, which may be in charge of certain biochemical activities, was found, according to the results. Ascorbic acid-like radical scavenging action was demonstrated using extracts. The antibacterial potential of the extracts was encouraging, both gram negative and gram-positive bacteria were suppressed by Triphala's active constituent. As a result, it has been concluded that triphala is a potential contender in green pharmaceuticals as a reproducible source of drugs for the future.

INTRODUCTION: The term "polyherbal formulations" (PHF) refers to medical formulations that contain many herbs.

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The idea of polyherbs has been around for more than a thousand years; predominantly observed in *Ayurveda* and diverse traditional medical system, where multiple herbs are taken in a certain proportion to cure the ailment and is believed to enhance therapeutic action and reduce adverse events [1]. The concept of "polyherbal formulations or polyherbalism," which aids in achieving greater therapeutic efficacy, was clarified by the ayurvedic literature "Sharangdhar Samhita" [2]. Individual plant's active phytochemical components fall short

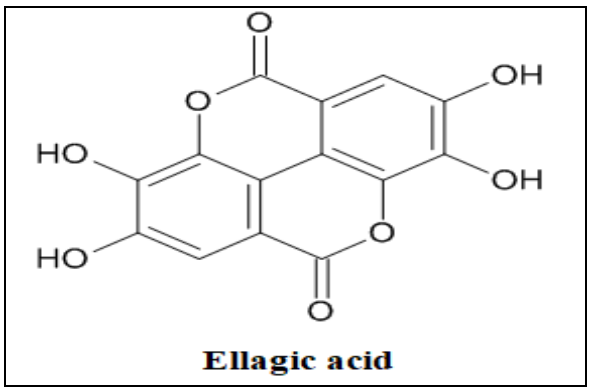
of providing the required therapeutic outcome. Polyherbal medicines are employed to address a range of conditions, including diabetes [3-4], cardiometabolic problems [5], kidney problem [6], hypertension, liver and skin conditions [7-8]. They are recognized to have antioxidant properties and reduced concentrations of single herbs, thereby reducing the risk of adverse events. Polyherbal drugs are composed of several herbs that work together to promote health and well-being, and may be used to supplement or replace single herbal remedies. Numerous plants have a higher medical effect and help to reduce toxicity when combined in a certain ratio [9].

Evaluation of polyherbal formulations is crucial to support their effectiveness, acceptability, sufficiency, and safety for usage. To evaluate the caliber of the drug's active ingredient, polyherbal expression standardization is crucial. Its foundation is an evaluation of its medicinal components, as well as its physical, chemical, and phytochemical qualities, as well as *in-vitro* and *in-vivo* standards [10].

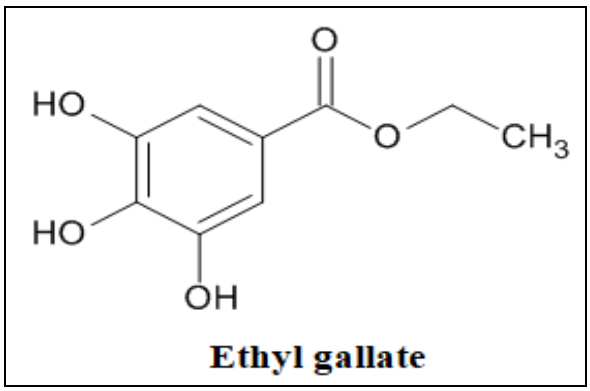
Significance of Polyherbal Formulations: Polyherbal remedies have played a key part in the treatment of both serious and minor medical disorders. The following text emphasizes the importance of polyherbal medicines' therapeutic potential.

- They have an expansive customer base.
- They offer a more compassionate experience.
- With the expansion in the field of knowledge and modern technology, the calibre, excellence, potential and well-being of herbal pharmaceuticals have been enhanced.
- Polyherbal are more affordable than other medications.
- They don't appear to cause any toxic effects or harm.
- Prolonged utilization of PHF may confirm its effectiveness and safety.
- Medicinal herbs or plants are known to be sustainable source of medicines [11].

***Triphala Churna*:** Originating in the region of the Indian subcontinent, triphala is a well-known and powerful polyherbal remedy made from the desiccated fruits of three different plant species: *Phyllanthus Emblica (F. Euphorbiaceae), Terminalia chebula (F. Combretaceae)* and *Terminalia bellerica (F. Combretaceae). Ayurveda* classifies triphala churna as a tridosha or tridoshic rasayana because it improves the life span, also revitalize individuals of all ages. According to "The Ayurvedic Pharmacopoeia of India," the composition contains dried form of three herbal fruits, Amalaki (Indian Gooseberry), Haritaki (Indian Hog Plum), and Bibhitaki (Vibheetaki) in 1/3 proportions each. It has been used for millennia in Indian medicine [12]. Triphala Churna has a variety of health benefits including- regulation of blood sugar levels, proper digestion, weight reduction, aiding inflammation, lowering cholesterol levels, normalizing blood pressure and improving circulation. It has antioxidant, anti-erythrogenic and antimicrobial properties (as shown in **Fig. 1** [13-18]. Triphala Churna is also known for promoting proper digestion and absorption of food, treating stomach ulcers, healing skin conditions, and treating arthritis and gout [19-26].



Ellagic acid



Ethyl gallate

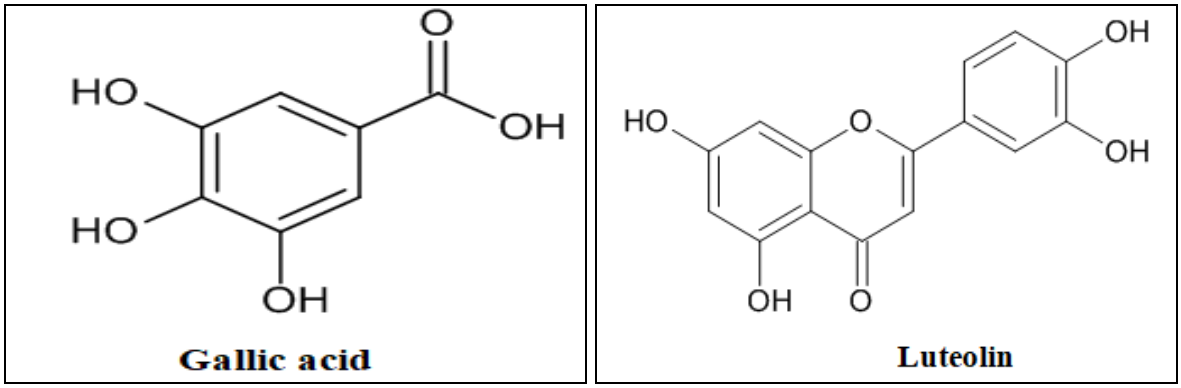


FIG. 1: SOME COMPOUND OF POLYHERBAL DRUG TRIPHALA CHURNA KNOWN FOR ANTIOXIDANT PROPERTIES

With the knowledge of triphala many therapeutic benefits, it has been suggested that we focus our research on phytochemical analyses of this understudied formulation and examine its therapeutic capabilities with scientific evidences. As a result, the triphala formulation was prepared using 3 different components. The phytochemical of herbal drug triphala were extracted using different solvents *i.e.*, Ethanol extract of Triphala (EET) and aqueous extract of Triphala (AET) and following, In-vitro antibacterial and antioxidant capabilities were also examined.

MATERIAL AND METHODS:

Procurement of Sample: In the current investigation, components of Laboratory prepared Triphala, which are Amalaki (Indian Gooseberry), Haritaki (Indian Hog Plum), and Bibhitaki (Vibheetaki), were bought from an authorized Ayurvedic shop situated in Muzaffarnagar's local market, as shown in **Fig. 2** and given them voucher sample no. A001, H002 and B003 all voucher samples have been authenticated by Professor of college as Botanist.

FIG. 2: CRUDE DRUG SPECIMENS OF AMALAKI (A), HARITAKI (B) AND BIBHITAKI (C) OBTAINED FROM AYURVEDIC SHOP

Procedure for Composing Triphala Churna: The unprocessed herbs bought for Laboratory prepare Triphala, were left to dry in shade at room temperature. Then, each herb was individually pulverized and put through sieves, the crude pharmaceuticals are then turned into a fine powder as illustrated in **Fig. 3**.

Which is then carefully combined in a 1:1:1 ratio in accordance with pharmacopoeial standards before being kept in an airtight container. After that, the preparation was subjected to multiple quality assurance checks.

FIG. 3: LABORATORY FORMULATION OF POLYHERBAL DRUG TRIPHALA CHURNA AFTER DRYING AND PULVERIZING THE UNPROCESSED HERBS (FRUITS) OF AMALAKI, HARITAKI AND BIBHITAKI

Chemicals and Test Microorganisms: All of the compounds utilized in the investigation were of the analytical variety. Sri Ram Scientific Co., Muzaffarnagar 251001, India, provided the analytical quality solvents and chemicals required for the phytochemical analysis, antioxidant, and antibacterial experiments.

The identified and acquired bacterial strains for the tests came from National Centre For Cell Science, Pune, India. These includes *Bacillus subtilis* (MTCC 2010), *Escherichia coli* (MTCC 3099), *Pseudomonas aeruginosa* (MTCC 2265) and *Staphylococcus aureus* (MTCC 2408).

Establishing Criteria for Standardization and Quality Attributes Parameters for Polyherbal Drugs:

Physico-chemical Parameters Determination: Physiochemical analysis is a method of analyzing a substance to determine its physical and chemical properties *i.e.,* to identify and quantify the various components of a substance, and to determine its overall purity and stability. The sample was evaluated for its acid- insoluble ash determination, water soluble ash determination, total ash determination, foreign organic matter, moisture content determination and ethanol soluble extractive value.

Pharmaceutical Evaluation of Churna: Pharmaceutical analysis involves the study of the chemical and physical properties of plant-derived substances that are used in the pharmaceutical field. This includes numerous identification and quantification test. The pharmaceutical test here, focused on the flowable properties of the plant material. The sample was evaluated for itsapparent and tapped density, carr's compressibility index, angle of repose and hausner's ratio.

Phytochemical Analysis: Phytochemical evaluation tests reveal variety of powerful bioactive compounds or substances that may be the cause of their therapeutic qualities can be seen and confirmed by phytochemical evaluations. Following the preparation of aqueous extract and ethanolic extract of laboratory prepared polyherbal drug, preliminary phytochemical testing was carried out on the substance. This test represents detection for Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Protein, Flavonoids, Phenol and Amino Acid.

Determination of Antibacterial Activity: The aqueous and ethanolic extract's antimicrobial activity was evaluated using the Shamsi et al. technique against Escherichia coli and Pseudomonas aeruginosa; gram-negative bacteria's and Bacillus subtilis and Staphylococcus aureus; gram-positive bacteria. An isolated colony from agar plates was used to inoculate Luria broth (LB) medium to create overnight cultures, which were then cultured at 37° C for 12 hours. In order to compare the growth of the overnight cultures to the control culture, which included just media and bacterial inoculum, using the fresh LB medium, the nocturnal cultures were diluted to roughly 104 colony forming units and then, at 37° C incubated for 12–14 h. For confirmation, the experiment was conducted twice more. The formula used to compute the percentage mean growth inhibition (%MGI) is as follows:

Formula: %MGI = dc-dt / dc × 100

Determination of Antioxidant Activity:

DPPH free Radical Scavenging Method: On the basis of the stable DPPH's ability to scavenge free radicals, the antioxidant activity of polyherbal drug, its components, and ascorbic acid (as standard) were assessed using a slightly modified version of the Braca *et al.* technique, provided by R. Parveen *et al.* Numerous diluted solutions of the polyherbal drug in aqueous and ethanol solutions were prepared, respectively. Standardization was done using distilled water and ascorbic acid (1 mg/ml). 500 ml of the 0.1 mM DPPH solution was combined with 500 ml of the standard solution and working sample solutions separately.

These solutions were stored in dark place. After 30 minutes of darkness, the optical densities of these solution combinations were measured at 517 nm with a spectrophotometer. The control was a solution of 0.1 mM DPPH. As a control, a variety of diluted aqueous and ethanolic extracts were used. The optical density was measured, and the following formula was used to calculated DPPH free radical scavenging is given below. Where dc and dt, respectively, stand for the control sample's and the test sample's absorbance at 517 nm.

Formula: DPPH scavenging activity (%) dc –dt / dc × 100

FRAP Assay: For FRAP assay, Minor adjustments were made by R. Parveen et al. to the ferric reducing antioxidant power (FRAP) technique developed by Benzie & Strain in order to conduct an antioxidant activity assessment.

In order to make the FRAP reagents, 50 ml of acetate buffer (0.3M) at pH 3.6, 5 ml of TPTZ solution 10mM prepared in HCl (40mM), and 5 ml of FeCl3 (20 mM) water solution were combined. In distilled water and ethanol, a variety of diluted working solutions of the Triphala and its component plants were created. After mixing each sample (200 ml) with 1.5 ml of newly made FRAP solution for 5 minutes, absorbance was recorded at 587 nm with FRAP working solution used as a reference. Standardization was done using ascorbic acid. The findings of both aqueous and ethanolic extract were expressed using mM Fe2+/ml. A higher absorbance corresponds to a stronger reducing power [27-28].

RESULTS AND DISCUSSION:

Physico-chemical Analysis: The findings of Physico-chemical analysis of polyherbal drug formulation were encouraging. The results obtained were within the limits of WHO and Indian Pharmacopoeia. The results for triphala preparations are reported in **Table 1.**

TABLE 1: OUTCOMES FOR PHYSICO-CHEMICAL EVALUATION FOR LABORATORY FORMULATION PREPARATION OF POLYHERBAL DRUG TRIPHALA CHURNA

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	44.6 ± 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: The findings of triphala's pharmaceutical analysis revealed that the polyherbal drug have poor flowable properties, it reported the apparent density of 0.391 and tapped density 0.539 respectively. According to carr's index (27.45) and Hausner ratio (1.37) it reported to have poor to very-poor flow properties, and shows a passable sign via angle of repose. The results obtained for triphala preparation are reported in **Table 2**.

TABLE 2: OUTCOMES FOR PHARMACEUTICAL ANALYSIS OF COMMERCIALLY AVAILABLE AND LABORATORY PREPARATION OF TRIPHALA CHURNA

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 findings of phytochemical assessment of AET and EET, revealed presence of various phytoconstituent and was analyzed based on chromogenic reactions. Both AET and EET reported the presence of major phytoconstituents *viz*. tannins, saponins, steroids, phenol, and flavonoids. The ethanolic extract gave exceptional chromogenic reaction in comparison to aqueous extract, which indicates ethanol solvent extracted more phytoconstituents than aqueous solvent. The results obtained from AET and EET are reported in **Table 3.**

TABLE 3: OUTCOMES FOR PHYTOCHEMICAL ANALYSIS EVALUATION FOR LABORATORY FORMULATION PREPARATION OF POLYHERBAL DRUG TRIPHALA CHURNA

Phyto-constituents	Tests	Triphala Extracts	
		AET	EET
Alkaloids	Hager's test	-	-
	Mayer's test	-	-
	Wagner's test	-	-
Carbohydrate	Benedict's test	+	++
Tannins	Braymer's test	+	++
Saponins	Foam test	+	+
Steroids	Salkowski's test	+	++
Proteins	Biuret test	-	-
Amino acid	Ninhydrin test	-	-
Phenol	Lead tetra acetic acid Test	+	++
Flavonoids	Shinod's test	+	++

"++"; Indicates the presence appreciable amount. "+"; Indicates the presence moderate amount. "-"; Indicates the absence of phytoconstituent

Antibacterial Activity: By obtaining the % MGI by the polyherbal drug triphala, the antibacterial capabilities of the ethanolic (EET) and aqueous(AET) extract was evaluated using several bacterial strains. Results were comparable to those of ampicillin (beta-lactam antibiotic) (used as standard antibiotic). A positive control was performed using just bacterial cultures and medium added. The findings indicated that triphala had bacteriostatic properties *in-vitro*, which means that microorganism growth, was suppressed, the presence of both extracts, as seen in **Fig. 6.** As shown in **Fig. 5**, it was discovered that AET reported the maximum inhibitory action against *B. subtilis* (95.030 ± 0.411%), whereas it exhibited the minimum inhibition action against *P. aeruginosa* (36.446 ± 0.251%) in **Fig 4**. EET was shown to have the maximum inhibitory effect against *B. subtilis* in ethanolic extracts (82.226 ± 0.396%), whereas *P. aeruginosa* exhibited the least inhibition (52.241 ± 0.411%). Ampicillin had the greatest effect on *E. coli* (MGI% 98.204± 0.498) and the least effect on *B. subtilis* (MGI% 88.68 ± 0.478). The investigation and observations discussed above showed that EET had the notable inhibition against all bacterial strains used, making it the most effective antibacterial agent. Overall, it concludes that triphala works as a broad-spectrum antibacterial agent. As show in **Fig. 6**, Triphala shows a greater potentialin comparison of the gram-positive bacteria to gram-negative bacteria.

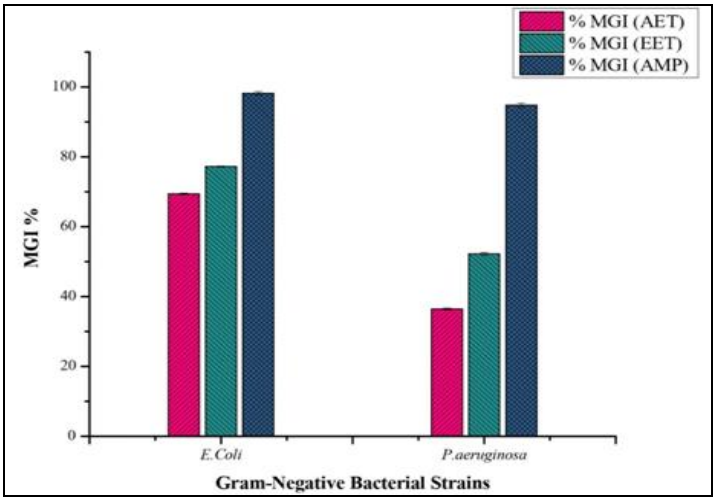


FIG. 4: DEPICTIONS OF IN-VITRO ANTIBACTERIAL ACTIVITY BY BROTH DILUTION TEST USING BAR DIAGRAM. THE BARS SHOW THE %MGI THAT TRIPHALA CHURNA EXTRACT WAS FOUND TO HAVE WHEN TESTED AGAINST TWO GRAM-NEGATIVE BACTERIAL STRAINS IN ETHANOLIC AND AQUEOUS EXTRACTS

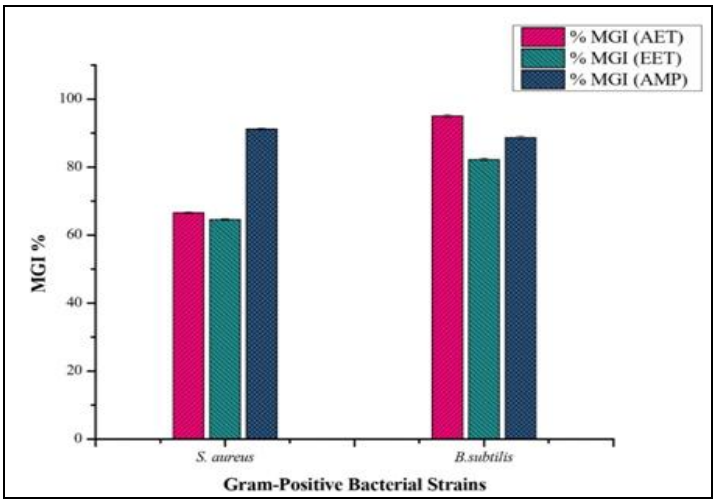


FIG. 5: DEPICTIONS OF IN-VITRO ANTIBACTERIAL ACTIVITY BY BROTH DILUTION TEST USING BAR DIAGRAM. THE BARS SHOW THE %MGI THAT TRIPHALA CHURNA EXTRACT WAS FOUND TO HAVE WHEN TESTED AGAINST TWO GRAM-POSITIVE BACTERIAL STRAINS IN ETHANOLIC AND AQUEOUS EXTRACTS

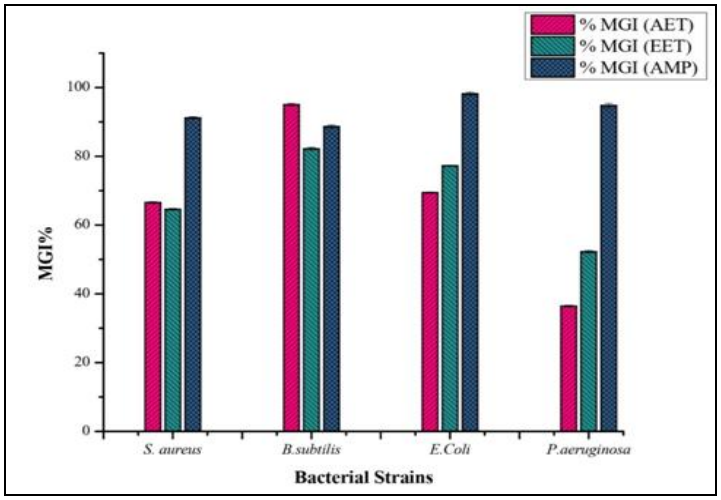


FIG. 6: DEPICTIONS OF COMPARISON OF IN-VITRO ANTIBACTERIAL ACTIVITY OF BOTH GRAM-NEGATIVE AND GRAM- POSITIVE BACTERIA BY BROTH DILUTION TEST, REPRESENTED USING BAR DIAGRAM. THE BARS SHOW THE %MGI THAT TRIPHALA CHURNA EXTRACT WAS FOUND TO HAVE WHEN TESTED USING ETHANOLIC AND AQUEOUS EXTRACTS

Antioxidant Activity:

DPPH: When the DPPH radical-scavenging capacity of the AET and EET were compared, both extracts demonstrated a considerable degree of DPPH scavenging capacity. The scavenging activities of each extract were compared to that of ascorbic acid. AET and EET, DPPH antioxidant activity measured and compared to ascorbic acid. The amount of extract that exhibited the highest and lowest levels of radical scavenging activity were 50 µL and 500µL, respectively. AET had the maximum DPPH free radical scavenging activity of 61.94± 0.42% and the minimum of 10.48± 0.25%. The DPPH radical scavenging activity of EET ranged from 19.18 ± 0.43% to 96.35 ± 0.37%. In comparison to ascorbic acid (Vitamin C), the graph showed that EET exhibits a large level of free radical scavenging activity, with a maximum DPPH activity of 93.39 ± 0.29% and a minimum of 84.35 ± 0.32%. The AET was closely followed by the EET in terms of DPPH scavenging as shown in **Fig. 7.**

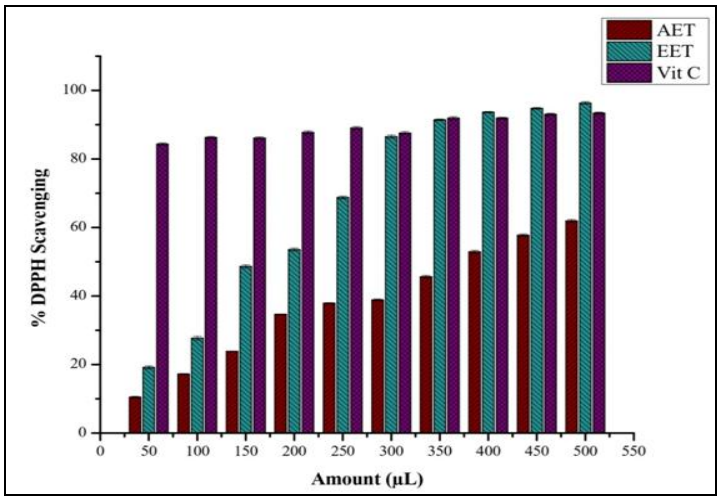


FIG. 7: DEPICTIONS OF COMPARISON OF ANTIOXIDANT ACTIVITY OF TRIPHALA'S ETHANOLIC AND AQUEOUS EXTRACT USING DPPH FREE RADICAL SCAVENGING METHOD REPRESENTED USING BAR DIAGRAM. ASCORBIC ACID WAS USED TO EVALUATE THE RESULTS

FRAP Assay: The FRAP test was used to gauge how well the plant extracts reduced ferric ions. The Fe (III)-TPTZ complex would be reduced into Fe (II)-TPTZ, which absorbs heavily at 593 nm, by an antioxidant capable of donating a single electron. Triphala demonstrated concentration-dependent FRAP antioxidant efficacy. For FRAP assay, the ethanolic and aqueous extracts were both examined. Results obtained were compared to the standard. The quantity of ascorbic acid and both

extracts, that demonstrated FRAP action in a concentration-dependent manner was 50 µL and 500 µL, respectively. As shown in **Fig. 8,** AET reported maximum values of 1.04 ± 0.018 mM and minimum values of 0.19 ± 0.003 mM and EET reported maximum value of 1.02 ± 0.016 mM and minimum value of 0.22± 0.002 mM. Observations indicate that triphala performed satisfactorily when compared to ascorbic acid. The maximum FRAP value was shownby AET followed by EET at 500µL extract.

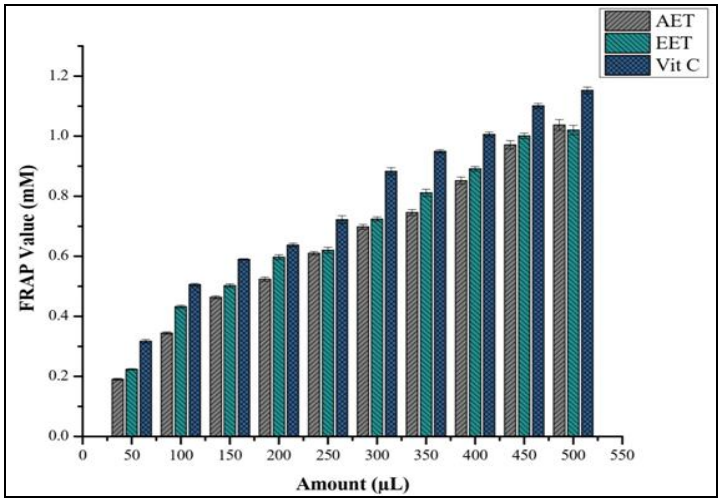


FIG. 8: DEPICTIONS OF COMPARISON OF ANTIOXIDANT ACTIVITY OF TRIPHALA'S ETHANOLIC AND AQUEOUS EXTRACT USING FRAP ASSAY METHOD USING BAR DIAGRAM. ASCORBIC ACID WAS USED TO EVALUATE THE RESULTS

CONCLUSION: This research explores, comparative analysis and a variety of features for standardization, including physico-chemical standards, pharmaceutical assessment, and phytochemical analysis. Furthermore, this research also concludes, triphala and its components have demonstrated potent antibacterial and antioxidant effects.

Consequently, it might be utilized as a possible source of organic antibacterial and antioxidant compounds. From the present investigation, it can be concluded that the plant may be examined for novel plant-derived chemicals that may be more effective antioxidants and antimicrobials.

In order to identify and separatebio-active molecules for a thorough assessment of *in-vivo* activity of such compounds, more investigation of triphala and its components is necessary.

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CONFLICTS OF INTEREST: The Authors declare no conflict of interests.

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