Phytochemical Scrutiny and antioxidant activity of Polyherbal using *Tricosanthes cucumerina, Terminalia chebula, Moringa oleifera,* and *Mangifera indica*

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ABSTRACT:

The current study was based on the analysis of qualitative phytochemical analysis and antioxidant activity in the polyherbal medicinal plant extract. The medicinal plants chosen for this study are *Tricosanthes cucumerina, Terminalia chebula, Moringa oleifera,* and *Mangifera indica.* Phytochemical components are highly present in plants and are highly used in pharmacological action on the human body. These are alternatives to synthetic drugs. Phytochemical analysis of primary metabolites, miscellaneous compounds, and secondary metabolites. Plant phytochemicals have higher pharmacological activity in the areas of antioxidant, anti-inflammatory, anti-allergic, antibiotic, hypoglycemic, anti-carcinogenic, and diabetes mellitus. Secondary metabolites with antioxidant properties are widely used to protect against free radicals. Fruits have high antioxidant properties when compared to vegetables. The antioxidant activity of the polyherbal extract is determined by the following methods: 2,2-di-phenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity assay, ferric reducing (FRAP) assay, and hydrogen peroxide (H_2O_2) assay.

Keywords: Phytochemical activity, Antioxidant activity, DPPH, FRAP, H_2O_2, Polyherbal.

INTRODUCTION

Medicinal plants offer potential for new herbaceous drugs and health management control due to their rich phytochemical elements and potential for future medicine. (Shakya & Correspondence, 2016)

Medicinal control in historic plant species relies on phytochemical factors, which have pharmacological activity
on the human body and can be divided into primary and secondary constituents. (Agidew., 2022)

Secondary metabolites, synthesized in various plant parts, are economically significant in drugs, fragrances, dyes, pesticides, food, and preservatives, with many medications derived from these secondary metabolites. (S Bansode & B K Salalkar, 2015). Secondary metabolism-originating compounds, particularly phenolic compounds, are crucial in combating oxidative stress and are commonly recognized as antioxidants due to their strength. (Chaves *et al.*, 2020)

The study uses standard tests to screen the phytochemical and antioxidant activity of polyherbal extracts from *Tricosanthes cucumerina, Terminalia chebula, Moringa oleifera,* and *Mangifera indica.*

Plant Description

Trichosanthes cucumerina, a popular plant with medicinal properties, is primarily consumed as a vegetable and has been used in ancient medicine for treating various ailments like antidiabetic, antibacterial, anti-angering, anthelmintic, antifebrile, gastroprotective, and antioxidant activity. (Liyanage *et al*., 2016)

Moringa oleifera, also known as the "Miracle Tree" or "Tree of Life," is used in India for medicine, treating conjunctivitis, and removing intestinal worms. The bioactive compounds present in *Moringa oleifera* confer properties associated with disorder prevention and treatment, such as antimicrobial, anti-inflammatory, anticancer, antidiabetic, antioxidant, hepatoprotective, and cardioprotective. (Milla *et al.,* 2021)

Mangifera indica L. Mango, the "king of fruits," is the national fruit of India, the Philippines, and Bangladesh, with its phytochemicals proving beneficial in counteracting pro-inflammatory and ROS production associated with human pathologies, such as cancer, cardiovascular disorders, aging, and neurodegenerative disorders. (Lauricella *et al.*, 2017)

Terminalia chebula, named the “King of Medicine” and known as haritaki, is a revered plant in Ayurvedic medicine, known for its medicinal properties and its potential to treat various health issues. It is a plant with anti-bacterial, antifungal, anti-malignant, antioxidant, antidiabetic, anti-inflammatory, anti-HIV, and anti-aging activities. (Bulbul *et al.*, 2022)

MATERIALS AND METHODS

Collection of Plant Materials

Tricosanthes cucumerina, Moringa oleifera, and *Mangifera indica* were collected from the local market of Coimbatore (Tamil Nadu, India). *Terminalia chebula* was collected from the herbal medicine shop in a local shop in Coimbatore (Tamil Nadu, India). The plant material was identified and its authenticity confirmed by comparing it with the voucher specimen at the herbarium of the Botanical Survey of India, Southern Circle, Coimbatore, India.

Sample preparation

The samples were washed with running tap water and distilled water, then spread on a white cloth to dry. The dried samples were powdered. (Zin, 2021)

Extraction Methods

The current study utilized a 50:50 ethanol:water extraction process, involving a 10g dried sample mixed with 50 ml of ethanol and 50 ml of water and stored at room temperature for 24 hours. The extract was then filtered through a Whatman filter paper. The extraction was stored for further activities. (Zin, 2021)

Extraction of *Trichosanthes cucumerina*

The extraction process involved adding 6g of dried sample to 60 ml of 50% ethanol, allowing it to stand at room temperature for 24 hours, and then filtering through Whatman filter paper. (Zin, 2021)

Extraction of *Moringa oleifera, Mangifera indica* and *Terminalia chebula*

At room temperature, 3g of dried *Moringa oleifera* was weighed, added to 50% ethanol to prepare a 30 ml extract, and allowed to stand for 24 hours before filtration. The same procedure was for the extraction of *Mangifera indica,* and *Terminalia chebula.* (Zin, 2021)

Preparation of polyherbal

A 70 ml polyherbal was prepared by adding 35 ml of *Trichosanthes cucumerina* in high concentration to 11.6

ml of each *Moringa oleifera, Mangifera indica,* and *Terminalia chebula,* mixed well, and stored for further analysis.

Qualitative phytochemical evaluation

Phytochemical screening of the polyherbal filtrate was assessed by standard methods as described by (Shaikh & Patil, 2020), (Baswal *et al.*, 2022), (Balamurugan Vishnu *et al.*, 2019) and (Kancherla *et al.*, 2019)

Primary Metabolites

Reducing Sugars (Benedict's test)

0.5 ml of Benedict's reagent was added to the 0.5 ml of filtrate. The mixture was heated for 2 minutes in a boiling water bath. The appearance of a red color indicates the presence of reducing sugar.

Carbohydrate (Molisch's test)

2 drops of Molisch's reagent were added to the 2 ml of filtrate. Shake well and add 2 ml of concentrated sulfuric acid along the sides of the test tube. At the junction of two solutions, a reddish-violet ring was formed, which indicates the presence of carbohydrate.

Starch (Iodine test)

2-3 ml of the filtrate were added to the 5 ml of distilled water, and 0.01g of iodine and 0.075g of potassium iodide were added. The formation of a blue color indicated the presence of starch.

Protein (Xanthoproteic Test)

2 ml of water and 0.5% of concentrated nitric acid were added to 2 ml of filtrate. The appearance of a yellow color indicates the presence of protein.

Amino Acid (Ninhydrin Test)

2 drops of ninhydrin reagent were added to 2 ml of filtrate. The appearance of purple a color indicates the presence of amino acids.

Miscellaneous compounds

Resin (Acetic Anhydride Test)

A few ml of acetic anhydride and 1 ml of concentrated sulfuric acid were added to 1 ml of filtrate. The color change from orange to yellow indicates the presence of resins.

Fixed oils and fats (Saponification Test)
A few drops of 0.5N alcoholic KOH and a drop of phenolphthalein were added to the few ml of filtrate and heated for 2 hours. The soap formation, or partial neutralization of alkali, shows the presence of fixed oils and fats.

Carboxylic acid (Effervescence Test)
1 ml of sodium bicarbonate solution was added to the 1 ml of extract. The production of effervescence indicates the presence of carboxylic acid.

Secondary Metabolites

Quinones (Sulphuric Acid Test)
1 ml of concentrated sulfuric acid was added to the 1 ml of filtrate. The appearance of a red color indicates the presence of quinones.

Alkaloids (Picric Acid Test)
3-4 drops of a 2% picric acid solution were added to the few ml of filtrate. The appearance of an orange color indicates the presence of alkaloids.

Glycosides (Borntrager's Test)
3 ml of chloroform is added to the 2 ml of filtrate and shaken well. The chloroform layer is separated, and a 10% ammonia solution is added. The appearance of a pink color indicates the presence of glycosides.

Phenols (Lead Acetate Test)
3 ml of a 10% lead acetate solution was added to the 5 ml of filtrate and mixed gently. The production of bulky white precipitates indicates the presence of phenols.

Tannins (Braymer's Test)
3 ml of distilled water and 3 drops of 10% ferric chloride solution were added to the 1 ml of filtrate. The appearance of a blue-green color indicates the presence of tannins.

Flavonoids (Lead Acetate Test)
A few drops of 10% lead acetate solution were added to the 1 ml of filtrate. The production of a yellow precipitate indicates the presence of flavonoids.

Polysterols (Acetic Anhydride Test)
2 ml of acetic anhydride and 2 ml of concentrated sulfuric acid were added to the 0.5 ml of filtrate. The color change from violet to blue or green indicates the presence of polysterols.

Saponins (Foam Test)
In a few ml of distilled water, the 0.5 mg of filtrate was added and shaken vigorously. The formation of froths indicates the presence of saponins.

Steroids (Salkowski's Test)
2 ml of chloroform and 2 ml of concentrated sulfuric acid were added to the 2 ml of filtrate. The appearance of red and yellowish green fluorescence indicates the presence of steroids.

Triterpenoids (Horizon's Test)
To 10 mg of filtrate, 1 ml of chloroform is added and mixed to dissolve it. 2 ml of concentrated sulfuric acid is added followed by 1 ml of acetic anhydride. The formation of a reddish violet color is positive for the presence of triterpenoids.

Terpenoids (Salkowski Test)
1 ml of chloroform and 1.5 ml of concentrated sulfuric acid along the sides of the test tube were added to 3 ml of filtrate. The appearance of a reddish-brown color indicates the presence of terpenoids.

Coumarins (NaOH Test)
3 ml of a 10% aqueous solution of NaOH is added to the 2 ml of filtrate. The appearance of a yellow color indicates the presence of coumarins.

Antioxidant Activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay
The antioxidant activity of the extracts was determined using the DPPH free radical scavenging assay. (Mahdi-Pour *et al.,* 2012). In a test tube, aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml of ascorbic acid were added as standard. In a test tube, aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml of sample were added, respectively. In all the test tubes, 0.5 ml of a 0.2 mg/ml solution of DPPH was added. The volume was made up to 3 ml with ethanol. The mixture was shaken vigorously and allowed to stand at room temperature, protected from light, for 30 minutes. (Kumara *et al.,* 2018). A control was prepared using 0.5 ml of the respective vehicle in place of filtrate or ascorbic acid. 80% ethanol was taken as a blank. Absorbance was measured immediately at 517 nm by a UV spectrophotometer (Mahdi-Pour *et al.,* 2012).

$$\% \text{ of Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of standard}}{\text{Absorbance of control}} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) Method:
The antioxidant activity of the extracts was determined using the Ferric Reducing Antioxidant Power (FRAP) Method. In a test tube, aliquots of 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml of sample were added, respectively. 3.8 ml of FRAP reagent was added. This reagent was previously prepared by mixing 10 parts of 300 mM sodium acetate buffer solution at pH 3.6, 1 part of 10 mM TPTZ, and 1 part of 20 mM $FeCl_3$ hexahydrate. The resulting mix was incubated for 30 minutes at 37 °C. The absorbance increase was measured at 593 nm in a UV spectrophotometer. A control was prepared using 0.5 ml of the respective vehicle in place of filtrate or ascorbic acid. Blank, reacted with distilled water (Chaves *et al.,* 2020)

$$\% \text{ of Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of standard}}{\text{Absorbance of control}} \times 100$$

Hydrogen Peroxide (H_2O_2) scavenging method:
The antioxidant activity of individual extracts was evaluated using the H_2O_2 method. 0.5 ml, 1.0 ml, 1.5 ml, 2.0 ml, and 2.5 ml of samples were added with 3.4 ml of 0.1 M phosphate buffer and 0.6 ml of 40 mM H_2O_2. This mixture was incubated for 10 minutes at room temperature. After incubation, absorbance was measured at λmax 230 nm against a blank solution. Ascorbic acid was used as a standard at different concentrations.

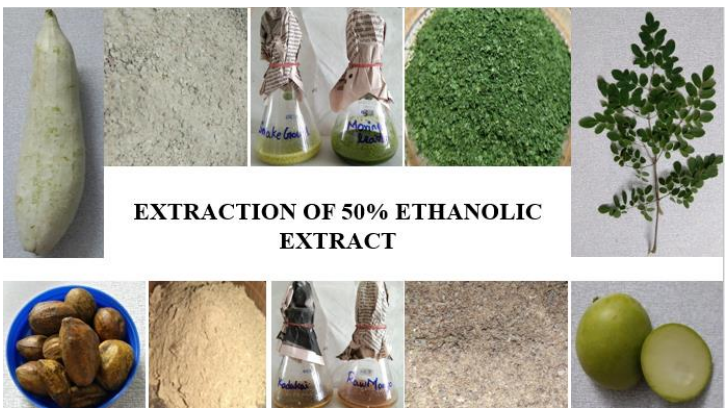
A control was prepared using 0.5 ml of the respective vehicle. The percentage scavenging of H2O2 was calculated using the equation. (Gupta *et al.*, 2022)

$$\% \text{ Percentage scavenging of } H_2O_2 = \frac{\text{Absorbance of control} - \text{Absorbance of standard}}{\text{Absorbance of control}} \times 100$$

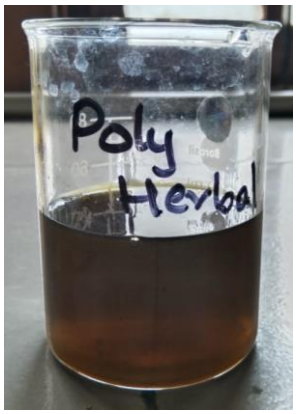
RESULT AND DISCUSSION

The plant extract was taken by using 50% ethanol. Polyherbal extraction was prepared using *Trichosanthes cucumerina, Moringa oleifera, Mangifera indica,* and *Terminalia chebula*. (Figure 1&2). Phytochemicals are plant-derived chemical compounds. The current study was carried out to find the presence and absence of reducing sugar, carbohydrate, starch, protein, amino acid, resin, fixed oils and fats, carboxylic acid, quinones, alkaloids, glycosides, phenols, tannins, flavonoids, polysterols, saponins, steroids, terpenoids, triterpenoids, and coumarins. The polyherbal 50% ethanolic filtrate shows that reducing sugar, carbohydrate, protein, amino acid, resin, carboxylic acid, quinones, alkaloids, phenols, tannins, flavonoids, polysterols, saponins, steroids, terpenoids, and coumarins are present. Starch, fixed oils and fats, glycosides, and triterpenoids are absent. (Tables 1&2).

Antioxidant percentage of inhibition increases as the concentration of the sample increases. Free radical scavenging by DPPH analysis. It shows that the polyherbal extract, when compared with standard ascorbic acid, exhibited 4.46%, 9.82%, 20.53%, and 31.25% at 10, 20, 30, and 40µg /ml concentrations and had a maximum inhibition of 50.89 % at 50 µg/ml concentrations of polyherbal extract. (Figure 3). Analysis by FRAP shows that the polyherbal extract, when compared with standard ascorbic acid, exhibited 18.09%, 26.66%, 39.04%, and 46.66% at 10, 20, 30, and 40 µg/ml concentrations and had a maximum inhibition of 62.85% at 50µg /ml concentrations of polyherbal extract. (Figure 4). Analysis by H_2O_2, shows that the polyherbal extract when compared with standard ascorbic acid exhibited 57.12%, 57.51%, 57.79%, and 58.94% at 5, 10, 15, and 20 µg/ml and have maximum inhibition of 59.33 % at 25 µg/ml concentrations of polyherbal extract. (Figure 5).



(Figure 1)
Trichosanthes cucumerina, Moringa oleifera, Terminalia chebula, and *Mangifera indica*



(Figure 2)
Polyherbal filtrate

Table 1&2. Qualitative phytochemical evaluation

(+ ; Presence, - ; Absence)

S.No	PHYTOCHEMICAL TEST	RESULT
SECONDARY METABOLITES		
1.	Quinones	+
2.	Alkaloids	+
3.	Glycosides	-
4.	Phenols	+
5.	Tannins	+
6.	Flavonoids	+
7.	Polysterols	+
8.	Saponins	+
9.	Steroids	+
10.	Terpenoids	+
11.	Triterpenoids	-
12.	coumarins	+

S.No	PHYTOCHEMICAL TEST	RESULT
PRIMARY METABOLITES		
1.	Reducing sugar	+
2.	Carbohydrate	+
3.	Starch	-
4.	Protein	+
5.	Amino Acid	+
MISCELLANEOUS COMPOUNDS		
1.	Resin	+
2.	Fixed oils & fats	-
3.	Carboxylic acid	+

Figure 3: Estimation of DPPH assay

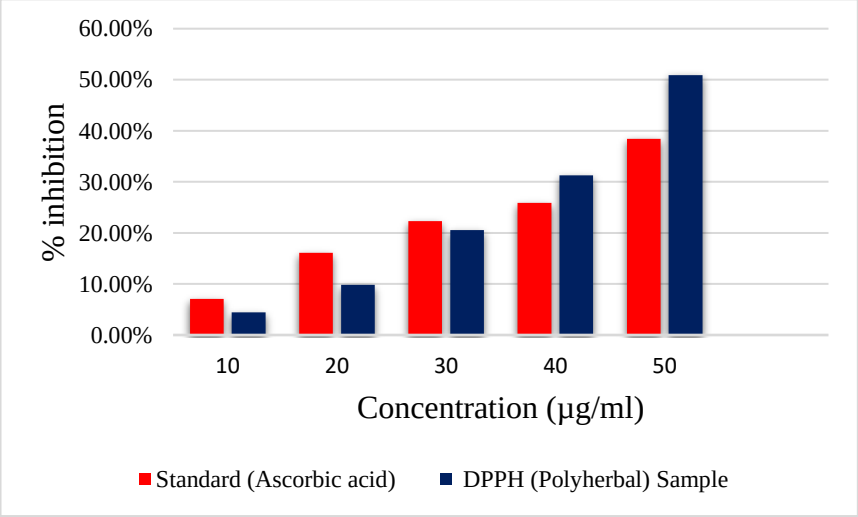


Figure 4: Estimation of FRAP assay

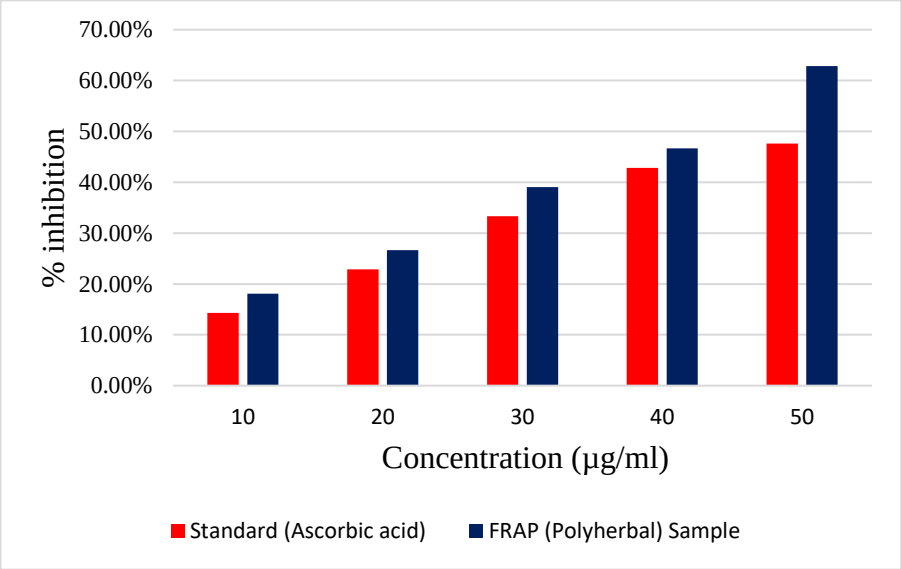
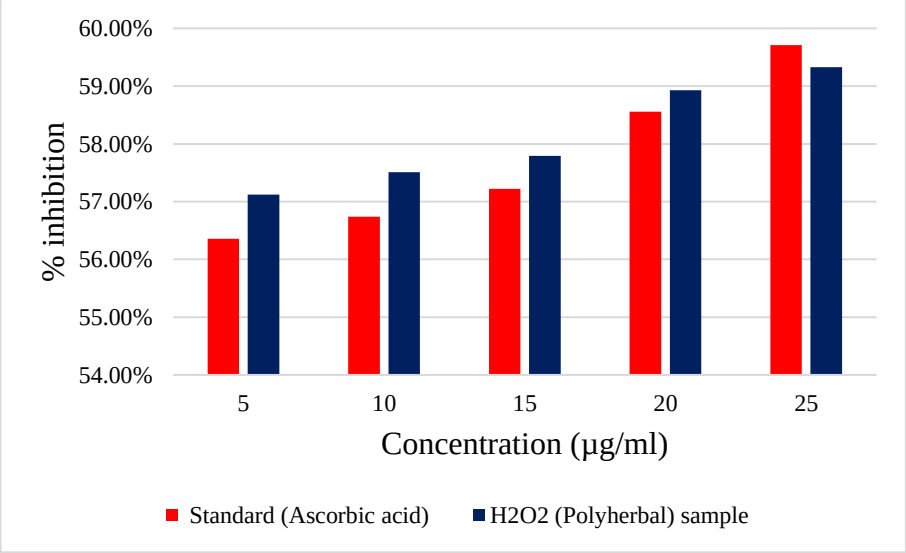


Figure 4: Estimation of H_2O_2 assay



CONCLUSION

The current study shows the presence of the phytochemical and antioxidant activity that is present in the polyherbal which is extracted from the 50% ethanolic extract. The presence of phytochemical components includes reducing sugar, carbohydrate, protein, amino acid, resin, carboxylic acid, quinones, alkaloids, phenols, tannins, flavonoids, polysterols, saponins, steroids, terpenoids, and coumarins. The free radical scavenging activity was shown by the DPPH method, the FRAP method, and the H_2O_2 method. Therefore, a future study can be done on the identification of the active components in the polyherbal extract.

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