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Research Article

Exploring the Synergistic potential of Polyherbal Extracts in Enhancing Antimicrobial and Antioxidant Activities

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ABSTRACT

Polyherbal formulations have gained significant interest due to their synergistic effects and therapeutic potential. This study aims to evaluate the phytochemical composition, antioxidant capacity, and antimicrobial activity of a polyherbal extract containing *Morinda pubescens*, *Terminalia catappa*, and *Tridax procumbens*. A qualitative phytochemical screening was performed to detect the presence of bioactive compounds, including flavonoids, phenolics, alkaloids, tannins, and saponins. The antioxidant activity was assessed through established *in vitro* assays, such as the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test and the ferric reducing antioxidant power (FRAP) assay. The results demonstrated a notable concentration of polyphenols and flavonoids, which correlated with significant antioxidant activity. Additionally, the antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria using the agar well diffusion method. The extract exhibited significant antimicrobial effects, inhibiting the growth of pathogenic microorganisms. These findings suggest that the polyherbal extract may offer potential health benefits, particularly in conditions associated with oxidative stress and inflammation, supporting its traditional applications in herbal medicine. Further research is warranted to explore its pharmacological potential and clinical applications.

INTRODUCTION

Phytochemicals, primarily derived from plant sources, are bioactive compounds classified as secondary metabolites. The economic significance of secondary metabolites is evident in their applications across various industries, including pharmaceuticals, flavourings, fragrances, dyes, pigments, pesticides, and food additives. Many pharmaceuticals are either synthetic modifications

or direct replicas of these naturally occurring substances (Twinkle S *et al.*, 2015).

Traditional medicinal plants, rich in phytochemical constituents, are widely used in formulations for skin-related ailments, particularly for wound healing (Bharadhan Bose *et al.*, 2024). Herbal remedies have shown potential in treating conditions such as ulcers, wound infections, skin

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diseases, inflammation, scabies, leprosy, and venereal diseases (Akshay Sharma *et al.*, 2021).

Antioxidants play a crucial role in enhancing quality of life by preventing or delaying the onset of degenerative diseases. Oxidative stress, a concept widely studied in medical sciences over the past three decades, is actively involved in the pathology of common diseases such as diabetes, hypertension, preeclampsia, atherosclerosis, acute renal failure, Alzheimer's disease, and Parkinson's disease (Nur Alam *et al.*, 2013). During oxygen metabolism, cells generate reactive oxygen species (ROS), which are potentially harmful. Antioxidants neutralize free radicals, preventing their negative impact on living organisms. In particular, antioxidants play a vital role in

counteracting oxidative stress. (Irina Georgiana *et al.*, 2021).

The concept of polyherbal formulations is well established and has achieved remarkable success in modern medicine, offering new hope to patients. In the pharmaceutical industry, several studies have demonstrated that combination therapy using plant extracts and antibiotics is more effective against diseases such as diabetes and cancer than monotherapy (Patel and Saravolatz, 2006). Polyherbal formulations were found to be more effective than individual constituents (Abd El-Kalek and Mohamed, 2012; Mundy *et al.*, 2016).

PLANT DESCRIPTION:

SPECIES NAME	COMMON NAME	PROPERTY
<i>Morinda pubescens</i>	Manjanathi, Noni or Indian mulberry	The leaves and root of <i>Morinda pubescens</i> are used as astringent and pain relievers in a acute inflammatory arthritis.
<i>Terminalia catappa</i>	country almond, Indian almond	It has various medicinal properties such as anti-inflammatory, antioxidant, anti-tumour, anti-HIV, and anti-diabetic
<i>Tridax Procumbent</i>	coatbuttons or tridax daisy	It's strongly proved for its Anti-inflammatory and Analgesic activity. It is also used as a treatment for boils, blisters, and cuts.

MATERIALS AND METHOD:

COLLECTION OF PLANT SAMPLES

The leaves of the plant samples (*Morinda pubescens*, *Terminalia catappa*, and *Tridax procumbens*) were collected from the local market region of Ganapathy, Coimbatore district 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 collected sample (*Morinda pubescens*, *Terminalia catappa*, and *Tridax procumbens*) were washed with a running tap water the plant material was dried by shaded dry for four days. The plant materials were coarsely powdered separately. By using a grinding miller to form fine solid. Somnath *et al.*, 2018

PREPARATION OF EXTRACT

The extraction process is done with the ethanol. To take 2g of each plant dried powder sample it treated with the 20ml ethanol. The mixture was allowed to stand for 24hours in a room temperature. The extract was then filtered through a Whatman filter paper. The extraction stored for further activities. Jaya patal *et al.*, 2023

PREPARATION OF POLY HERBAL EXTRACTS

Polyherbal extract was prepared by taking 3 g of extract of all plants, that is, *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbens* and properly mixing to get 180 ml of polyherbal extract stored for further analysis.

QUALITATIVE PHYTOCHEMICAL ANALYSIS



Phytochemical screening of the polyherbal extracts was assessed by standard methods **Tiwan et al., 2011 Swamini kute 2024**

- Test for alkaloids - Wager's test
- Test for flavonoids - Alkaline reagent test
- Test for Reducing sugar test – Fehling's test
- Test for Saponins - Foam test
- Test for phenol - Ferric chloride test
- Test for tannin - Ferric chloride test
- Test for terpenoids - Salkowki test
- Test for steroids - Salkowki test
- Test for protein - Ninhydrin test
- Test for glycosides

ANTIOXIDANT ACTIVITY

DPPH radical scavenging activity

Antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical inhibition assay. Several concentrations of compounds (10 to 50 μ l) were prepared for the sample Solution. To each sample solution 500 μ l of DPPH (100 μ g/mL) and 0.5mL of ethanol were added. The mixture was incubated for 30 min. The absorbance was measured at a wavelength of 517 nm **Kumar et al., 2011**. A control was prepared wing 0.5 ml of the respective vehicle in place of filtrate or ascorbic acid. 80% ethanol was taken as a hank. **(Mahdi-Pour et al.,2012)**. A plant extract with IC 50 <100 μ g/ml has a strong antioxidant activity. While one with IC 50 50mg/ml is considered a very strong antioxidant agent. **(Jumina et al., 2019)**

FRAP

The antioxidant activity of the plant extracts is determined using FRAP method. In a test tube, aliquots Several concentrations of compounds (10 to 50 μ l) were prepared for the sample were added respectively. 3.8ml of FRAP reagent were added. This reagent was previously prepared by mixing 10 parts of 300mM sodium acetate buffer solution at pH 3.6. 1 part of 10mM TPTZ and 1 part of 20mM FeCl₂ hexahydrate. The resulting mix was

incubated for 30 minutes at 37°C. The absorbance increase was measured at 593nm in a UV spectrophotometer. A control was prepared using 0.5ml of respective vehicle in place of filtrate or ascorbic acid. Blank reacted with distilled water. **(Chaves et al., 2020)**. A plant extract with IC 50<100 μ g/ml has a strong antioxidant activity. While one with IC 50<50mg/ml is considered a very strong antioxidant **(Jumina et al.,2019)**.

ANTI MICROBIAL ACTIVITY

Antibacterial Activity

Agar Well Diffusion method

The antibacterial activity of polyherbal extract was determined by Well Diffusion method **(Bauer et al., 1996)**. The 25 to 100 μ l of extract was poured into the wells. After that, the plates were incubated at 37°C for 24 hours. Assay was carried into triplicates and control plates were also maintained. Zone of inhibition was measured from the edge of the well to the zone in mm. The tested cell suspension was spread on potato dextrose agar. Well were put into the agar medium using sterile forceps. Plant extract were poured on to wells. Then plates were incubated at 37°C for about 24 hours and control was also maintained. Zone of inhibition was measured from the clear zone in mm.

Agar well diffusion method has been used to determine the antimicrobial activities and minimum inhibitory concentrations or plant extracts against Gram-positive, Gram-negative bacteria. The extracts exhibited antibacterial activities against tested microorganisms.

RESULT AND DISCUSSION

COLLECTION OF PLANT SAMPLE

The leaves of the plants *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbent (L)* were collected from the local market in the region of Ganapathy Coimbatore district

Morinda pubescens





Terminalia catappa

Tridax procumbent

SAMPLE PREPARATION

The samples were washed with distilled water and dried away from the sunlight. The dried samples were powdered.

PREPARATION OF EXTRACT

Ethanol is used for the sample extraction for 24 hours at room temperature using the maceration method. And the extracted sample was filtered. The extract was stored in a container in refrigerator for further use

PREPARATION OF POLY HERBAL EXTRACTS

Polyherbal extract was prepared by the ratio of 1:20 where the 3g of the each plant sample of *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbent* (L) in the 180ml of the ethanol as solvent.

QUALITATIVE

PHYTOCHEMICAL

ANALYSIS

The polyherbal extract of *Morinda pubescens*, *Terminalia catappa*, *Tridax 1080rocumbent* (L) were subjected to various qualitative phytochemical tests to determine the phytochemical constituents present in the extract

Phytochemicals	<i>Morinda pubescens</i>	<i>Terminalia catappa</i>	<i>Tridax procumbens</i>	Polyherbal extract
Alkaloids	+++	+++	++	+++
Flavonoids	++	++	+++	++
Reducing sugar	+	+	+	-
Saponin	-	+	+	-
Phenols	++	+++	++	++
Tannin	+++	++	+	+
Terpenoids	+++	+	++	+
Steroids	+	+	-	++
Protein	-	++	-	-
Glycosides	+	+	+	+++

+++” indicates strongly positive, “++” indicates positive, “-” indicates negative

ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS

Ethanolic extract of *Morinda Pubescens* was found to be contains flavonoids, Phenols, reducing sugar, tannin in higher content. Steroids, alkaloids, terpenoids, Saponin were found to be present in

leaf extract.

Ethanolic extract of *Terminalia catappa* was found to be contains alkaloids, flavonoids, Phenols, Saponins, Reducing sugar, tannin in higher content. Protein, steroids, terpenoids found to be present in leaves extract.

Ethanolic extract of *Tridax procumbent* was found to be contains flavonoids, Phenols, reducing sugar,

tannin in higher content. Saponins, steroids and terpenoids found to be present in leaf extract. Polyherbal leaves extract of *Morinda Pubescens*, *Terminalia catappa*, *Tridax procumbent*(L) was found to be contains the alkaloids, flavonoids, Phenols, reducing sugar, tannin In the higher content steroids, terpenoids are present. So the polyherbal leaf extract has been contains the most of the bio active component

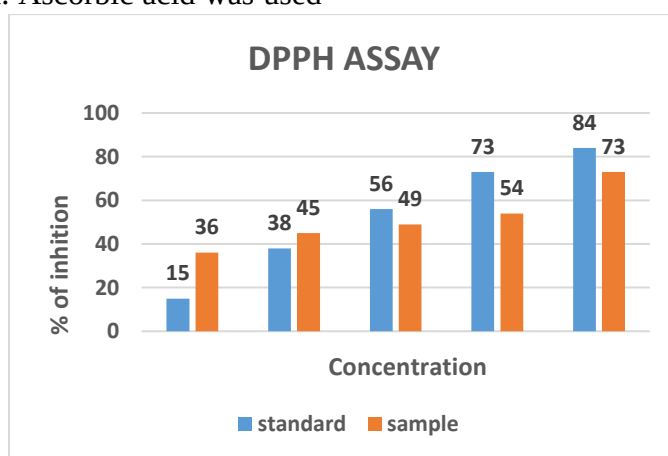
EVALUATION OF ANTIMICROBIAL ACTIVITY:

Evaluation of DPPH

The DPPH radical scavaging activity of polyherbal extract were investigated. Ascorbic acid was used

as a standard at concentration (10 – 50 µl/ml) it showed inhibitory activity from 15% to 84%. In the polyherbal extract at concentration (10-50µl) showed inhibitory activity from 36% to 71% respectively

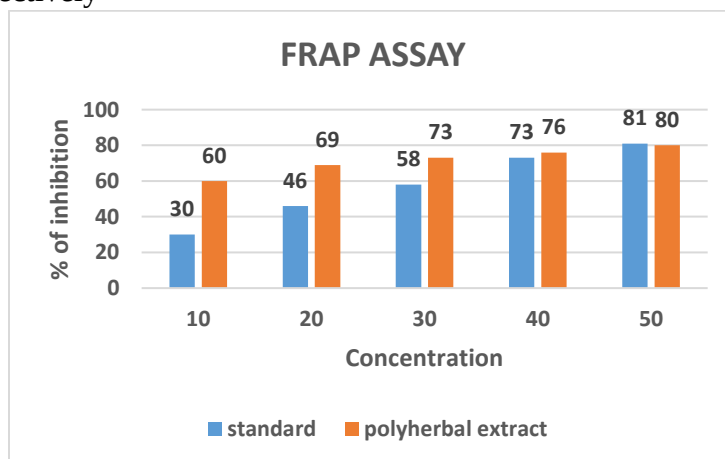
In In vitro antioxidant activity of polyherbal extract is observed at different concentrations. The result shows DPPH activity of the polyherbal extract and compared with each other with standard Ascorbic acid. It was found that polyherbal extract had the higher activity than the standard Ascorbic acid



Evaluation of FRAP assay

The FRAP radical scavenging activity of polyherbal extract were investigated. Ascorbic acid was used as a standard at concentration (10 – 50µl/ml) it showed inhibitory activity from 30% to 81%. In the ethanolic poly herbal extract at concentration (10-50µl) showed inhibitory activity from 60% to 80% respectively

The In vitro antioxidant activity of polyherbal extract is observed at different concentrations. The result shows FRAP activity of the polyherbal extract and compared with each other with standard Ascorbic acid. It was found that poly herbal extract had the similar activity



EVALUATION OF ANTI MICROBIAL ACTIVITY:

Agar well diffusion method

The antibacterial activity of ethanolic extract of *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbens* was found good against gram positive bacteria and gram negative bacteria shows the zone of inhibition against in both *Escherichia coli* and *Staphylococcus aureus*. The concentration of the sample was taken as 25,50,75,100 µl of respectively. The highest antibacterial activity is seen at the highest concentration of 100 µl of the

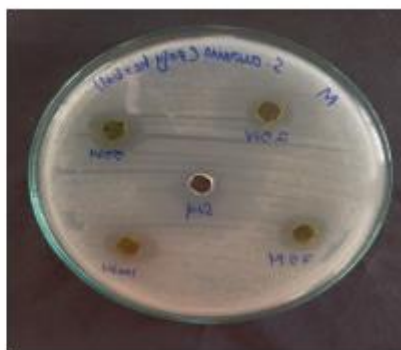
sample. The activity that is zone of inhibition increases with the increase in concentration of sample, shows that ethanolic extract of *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbens* potent antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, when compared with standard.

The agar diffusion a test of the antibiotic sensitivity of bacteria. It is widely used to determine the zone of inhibition of bacteria. Bacteria which are used to identify antimicrobial activity are *Escherichia coli*, *Streptococcus aureus*

Name of the organism	Sample 25µl	Sample 50µl	Sample 75µl	Sample 100µl	Standard
<i>Escherichia coli</i>	12mm	16mm	18mm	13mm	20mm
<i>Streptococcus aureus</i>	12mm	14mm	16mm	18mm	14mm



E.coli



S.aureus

CONCLUSION:

The polyherbal extracts of the *Morinda Pubescens*, *Terminalia catappa*, *Tridax procumbens* detected for the studying the phytochemical, Antioxidant, Antimicrobial, by using *In vitro* Methods. It was carried out by using the ethanol as solvent. The findings of the current study are summarised as follows:

Polyherbal leaves extract of *Morinda Pubescens*, *Terminalia catappa*, *Tridax procumbens* contains the alkaloids, flavonoids, Phenols, reducing sugar, tannin in the higher content steroids, terpenoids are present. so the polyherbal extract has been contains the most of the bio active component. Hence it' subjecte' to qualitative analysis and also used for current study.

The *in-vitro* free radicals scavenging effect is well observed in the of The polyherbal extracts of the

Morinda Pubescens, *Terminalia catappa*, *Tridax procumbens*. This implies that the extract is highly active of the scavenging activity the free radicals by annealing the proton thereby reducing the oxidative stress and the disorder and disease.

The polyherbal extract of *morinda pubescens*, *terminalia catappa*, *tridax procumbens*. Posses the good *in vitro* anti-bacterial activity it suppressing the growth of the gram positive and gram negative bacteria From the above finding it can be concluded that polyherbal extract of *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbens*. Shows the antioxidant, antibacterial, may be due to the presence of the phytoconstituents in the plant *Morinda pubescens*, *Terminalia catappa*, *Tridax procumbens*. can be used in treating the bacterial infection.

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