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Antioxidant Potential and Phytochemical Screening of a Polyherbal Formulation

N. Ramasamy[1], Saroj Yadav[2], S. Sri Bhuvaneswari[3], Ashish Chimbalkar[4], T. Sivakumar[5], Anagha Amit Sarvadnya[6], Suprodip Mandal[7], Mangirish Deshpande[8*]

[1]Associate Professor, Department of Pharmacognosy, S. A. Raja Pharmacy College, Vadakkangulam, Tirunelveli, Tamil Nadu, 627116, India

[2]Associate Professor, School of Medical and Allied Sciences, K.R. Mangalam University, Sohna Gurugram, Haryana, India

[3]Associate Professor, Department of Pharmaceutical Biotechnology, Nandha College of Pharmacy. Erode, 638052, Tamil Nadu

[4]Assistant Professor, Dr. D Y Patil College of Pharmacy, Pune, Maharashtra, India

[5]Principal, Nandha college of Pharmacy, Erode, Tamil Nadu, 638052, Affiliated to the TN Dr MGR Medical University, Chennai, India

[6]Assistant Professor, SMES'S Mahavir Institute of Pharmacy Nashik, Maharashtra, 422008, India

[7]Associate Professor, Department of Pharmacognosy, Bharat Technology, Howrah, West Bengal, 711316, India

[8]Associate Professor, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi Ponda, North Goa, 403401, India

***Corresponding author: Mangirish Deshpande,** Associate Professor, PES's Rajaram and Tarabai Bandekar College of Pharmacy, Farmagudi Ponda, North Goa, 403401, India

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ABSTRACT:

Plants are the best place to get antioxidants, which can help reduce the damage that free radicals do to cells. The goal of this study is to use an in vitro method to find the best phytochemical and antioxidant characteristics of polyherbal mixtures made from the methanolic extract of Phyllanthus emblica, Moringa oleifera, and Citrus limon leaves. Phytochemical testing showed that the different products contained flavonoids, alkaloids, glycosides, carbohydrates, amino acids, tannins, steroids, and steroids. The DPPH scavenging experiment was used to test the antioxidant activity of the different extracts and the mixture of herbs. When two or more extracts work together, they create an effect that is stronger than the sum of their individual effects. This is called synergy. Based on the results, P. emblica may be able to fight free radicals better. The multiple herbs worked together to make them more effective. The findings showed that the mixtures made for this study support the polyherbal mixtures' powerful antioxidant qualities. Based on the findings, P. emblica may have better antioxidant activity than other extracts. The multiple herbs worked together to make them more effective.

Keywords: *Antioxidant potential, phytochemical screening, polyherbal formulation*

INTRODUCTION:

It looks like natural mending methods are the only way to deal with modern problems and live a healthy life. The most effective method is Ayurveda, which uses a variety of natural phytoconstituents to get rid of serious illnesses [1, 2] by restoring balance and stopping them from happening again. The WHO says that about 80% of the world's population still relies on traditional or Ayurvedic medicines for their health and well-being [1-3].

Indians created the Ayurvedic medicine system to help people with their problems and keep them from having to suffer needlessly. It is known for having a lot of different kinds of plants, with more than 45,000 different kinds of herbs [2-4]. Combining two or more different herbal therapies with about 15,000 different medicinal herbs has been shown to help people with a wide range of diseases. Medicinal plants have been used by people since ancient times and are a natural gift to us. Bioactive chemicals that are naturally found in veggies, herbs, and other plants are thought to have many health benefits [3-5].

The powerful bioactive ingredients of individual plants are not enough to achieve the desired treatment effectiveness. Combining several herbal plants (polyherbal) in the right amounts would have a big impact on healing. Polyherbal preparations, which are made up of two or more plants with different bioactive compounds and healing qualities, have been shown to help people who are sick [4-6]. Polyherbal mixtures are popular because they have a wide treatment window, meaning they are safe at high doses and effective at low doses while also having few side effects. Ayurvedic medicine uses the whole plant, including the fruit, leaves, flowers, juice, bark, stem, gum, seeds, and roots. Since ancient times, people have been interested in many official herbs as natural sources of medicines [5-7].

People believe that vitamin C and flavonoids are good for your health because they naturally contain antioxidants. Because they are high in flavonoids, lemon fruits are an important part of a healthy diet because they may help avoid diabetes, obesity, dyslipidemia, heart disease, and some cancers. Moringa oleifera is in the Moringaceae family, which has about 13 other species [6-8]. It can be found in the Caribbean Islands, the Philippines, Cambodia, Central America, North America, and South America. Africa, Asia Minor, and the Indian region are where it comes from. The drumstick tree, or M. oleifera Lam, is the most well-known species [7-9]. Different parts of M. oleifera are used for many things, like cleaning water, making fertilizer and green manure, feeding animals, making medicines, using as biopesticides and nutrients for plants, and getting vitamin *C. Moringa* seeds are used to clean and disinfect water that is very cloudy [8-10].

They have been looked at to see if they could be used as different treatments for a number of diseases caused by the harmful effects of oxidants. There are many substances in these plant extracts that act as strong antioxidants [11-16]. The goal of this study is to find out how the leaf extracts of *M. oleifera, P. emblica*, and *C. limon* interact with each other to affect antioxidant activity. This will be done after looking at how they work together in a polyherbal product.

MATERIAL AND METHODS:

Materials:

Plant Materials:

We got leaves from *M. oleifera*, *P. emblica*, and *C. limon* at a number of places in India. After the medicines

were ground up in a Wilay mill, methanol was used as the solvent in a Soxhlet system to get them out.

Plant extracts:

Powdered *M. oleifera, P. emblica*, and *C. limon* plants were dried in the shade at room temperature. The plants that had been dried in the shade were broken up into a coarse powder, and then petroleum ether was used to remove the oils from the powder. The process of separation kept going until the material was fat-free. Before they were used, 100 grams of dried leaves from *M. oleifera, P. emblica*, and *C. limon* were fully extracted with methanol over the course of 48 hours [17-19]. The leaves were then filtered and dried at 400 degrees Celsius in a vacuum evaporator. After that, they were put away in a container that could not leak and was free of any pollution. It was worked out what the yield ratios of the dried extracts were. When 25 mg of each ingredient was mixed together, the mixture was called a polyherbal formulation. The solution was heated in a water bath for five minutes, cooled, and then centrifuged at 4000 rpm for ten minutes. This was done after dissolving 25 mg of leftover from each mixture in 10 ml of methanol. A number of tests were done to look at the clear supernatant's antioxidant properties [20-23].

Phytochemical Screening:

Standard methods were used for phytochemical screening to find bioactive chemicals. The results of the tests were based on what happened to the solution's color or the formation of precipitates when certain chemicals were added [24-27].

DPPH antioxidant activity:

To test the DPPH scavenging action, a different method was used. The spectrophotometer was used to measure how much DPPH was being scavenged. To get a starting absorption, 1.5 ml of the solution was mixed with 1.5 ml of methanol to make a stock solution (6 mg in 100 ml of methanol). When sample extract at different amounts was added, there was a drop in absorbance after 15 minutes. After getting 1.5 ml of DPPH solution and diluting it with methanol to make 3 ml, absorbance was tested right away at 517 nm for the control reading [28-32]. A set of volumetric flasks were each filled with 1.5 ml of DPPH and 1.5 ml of the test substance in a range of amounts. The end volume was changed to 3 ml with methanol. Three test samples were collected and handled in the same way. In the end, the average was found. At 0 time, the absorbance was recorded for each concentration. A further drop in DPPH absorbance was seen with the sample at different amounts after 15 minutes at 517 nm. As the IC50 number goes down, the antioxidant activity goes up [32-35].

RESULTS AND DISCUSSION:

Plant extracts:

The process of separation kept going until the material was fat-free. Before they were used, 100 grams of dried leaves from *M. oleifera, P. emblica*, and *C. limon* were carefully extracted with methanol over the course of 48 hours, filtered, and then dried in a vacuum evaporator at 400 degrees Celsius. After that, they were put away in a container that could not leak and was free of any pollution [36-38]. It was worked out what the yield ratios of the dried extracts were. After the maceration extraction method, the crude extracts were concentrated in a water bath to get rid of all the solvents and find out the extraction yield. The extract

yield is shown as a fraction in table 1 and Figure 1.

Table 1: Percentage of Methanolic Extract Yield

Sr. No.	Extracts Taken	% Yield
1	*Phyllanthus emblica* extract	6.12
2	*Citrus limon* extract	8.34
3	*Moringa oleifera* extract	7.51

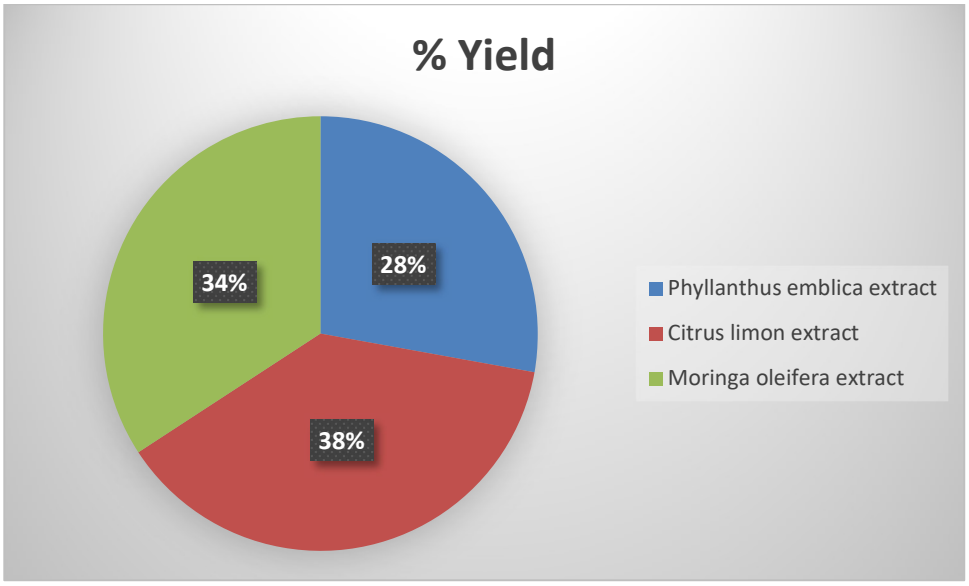

Figure 1: Percentage of Methanolic Extract Yield

Phytochemical Screening:

The results of the tests were based on what happened to the solution's color or the formation of precipitates when certain chemicals were added [39-43]. According to Table 2, the phytochemical study of plant methanolic extracts did not find any proteins, glycosides, oils, or lipids. However, flavonoids, phenols, alkaloids, carbohydrates, and saponins were found.

Table 2: Methanol extract phytochemical screening results

S. No.	Constituents	*P. emblica*	*M. oleifera*	*C. limon*
1.	Phenolics	+	+	+
2.	Amino Acids	+	-	-
3.	Proteins	+	-	-
4.	Flavonoids	+	+	+

5.	Alkaloids	+	-v	+
6.	Diterpenes	+	+	+
7.	Glycosides	-	-	-
8.	Oils and fats	-	-	-

+ Present, - Absent

DPPH antioxidant activity:

To test the DPPH scavenging action, a different method was used. The spectrophotometer was used to measure how well the compound removed DPPH. To get a starting absorbance, 1.5 ml of the solution was mixed with 1.5 ml of methanol to make a stock solution. When sample extracts at different amounts were present for 15 minutes, the absorbance went down. After getting 1.5 ml of DPPH solution and diluting it with methanol to make 3 ml, absorbance was tested right away at 517 nm for the control reading [28-30]. A set of volumetric flasks were each filled with 1.5 ml of DPPH and 1.5 ml of the test substance in a range of amounts. To get a finished volume of 3 ml, methanol was used. The DPPH test was used to measure the antioxidant activity of the samples. The amount of inhibition was found to show how strong the antioxidant was. The percentage of inhibition goes up as the movement gets stronger. Ascorbic acid concentrations as a standard ranged from 50 µg/ml to 300 µg/ml, and the data stayed the same [44-48]. Table 3 showed that the concentration had an effect that changed with amount.

Table 3: % DPPH inhibition of ascorbic acid, methanolic extract, polyherbal formulation

Sr. No.	**Conc. (µg/ml)**	***P. emblica* % Inhibition**	***P. emblica* % Inhibition**	**Ascorbic acid % Inhibition**	***C. limon* % Inhibition**	**Polyherbal F % Inhibition**
1	50	20.22±0.42	17.33±1.35	20.75±0.98	21.50±1.41	24.65±0.60
2	100	22.33±0.51	21.66±1.82	32.70±1.45	25.20±1.22	31.30±1.20
3	150	31.25±0.33	25.82±1.66	45.82±0.86	31.25±1.55	44.55±0.78
4	200	42.28±0.63	37.54±1.34	55.82±1.75	44.49±0.78	57.50±1.23
5	250	50.82±0.89	50.21±1.59	70.61±1.55	54.50±0.60	64.98±1.36
6	300	65.26±1.18	61.68±1.34	80.75±0.84	61.80±1.20	73.45±1.78

CONCLUSION:

The phytochemical analysis gave useful information about the different phytoconstituents found in the plant. This will help future researchers choose the right sample to study further to find the active ingredient. It also gave information about the different phytochemicals that were found to have different effects. The multiherbal mixtures used in this study worked together to effectively remove DPPH radicals through methanolic extracts. In the future, this finding could help people make better products, especially ones that are easier to understand,

especially ones that are about the plants being studied.

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Conflict of Interest:

None

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