EVALUATION OF SYNERGISTIC POTENTIAL OF COMBINED EXTRACTS OF APPLE PEEL AND GOLDENSEAL ROOT EXTRACT

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Abstract

The study investigates the combined effects of apple peel and goldenseal root extracts as antioxidant and antimicrobial agents. Aqueous and methanolic extracts were found to be the most potent, so two combinations were prepared: methanol combined extracts and aqueous combined extracts. These were used for antioxidant assays such as DPPH radical scavenging, ABTS assay, lipid peroxidation inhibition, and peroxynitrite bleaching of Pyrogallol red. Additionally, antimicrobial assays, including disc diffusion methods, were conducted against various gram-positive and gram-negative bacteria. The study aims to evaluate the synergistic potential of these combined extracts.

Introduction

Herbal medicines, an alternative to chemical remedies, involve the use of plant-based products to restore health. According to the EU, herbal medicinal products are derived from plant materials or preparations containing active ingredients. The process of converting botanicals into drugs requires careful quality control and standardization, combining traditional knowledge with modern technology. Countries like India and China have long traditions of herbal medicine, while other nations, such as the UK and Germany, incorporate folk medicine alongside modern practices. Even countries like Canada and Australia, which primarily use allopathic medicine, have recognized the need to regulate herbal remedies due to their growing use among immigrant populations.

Herbal remedies consist of crude plant preparations, including aerial or underground parts of plants, and can take various forms such as extracts, tinctures, or essential oils. The World Health Organization (WHO) has recognized the widespread use of herbal medicine, with 80% of the global population relying on it for primary healthcare. However, challenges remain, such as the lack of standardized quality control for herbal products. WHO guidelines emphasize the need for proper identification, safety, and effectiveness of herbal medicines.

As interest in herbal remedies grows globally, there is a pressing need for internationally recognized guidelines to ensure the quality and safety of these products. This includes advanced analytical techniques to address the complexity of plant-based components. In India, traditional plant-based medicines are widely used, with more than 1.5 million practitioners and significant market growth in Ayurvedic medicines.

Plants not only provide therapeutic benefits but also serve as models for drug synthesis. The use of herbal products in cosmetics, health supplements, and pharmaceuticals continues to expand, contributing to both healthcare and economic growth. To ensure the reproducibility and safety of herbal medicines, comprehensive research, quality

control, and regulatory measures are essential. The synergistic potential of plant extracts for antimicrobial and antioxidant activities has gained attention in recent years. Apple peel and goldenseal root are known for their individual therapeutic properties, with apple peel being rich in polyphenols and flavonoids, while goldenseal root contains berberine, an alkaloid with notable antimicrobial activity. The study aims to evaluate the combined effects of these two extracts on enhancing antioxidant and antimicrobial properties. By combining the extracts, researchers hope to leverage their synergistic action, resulting in a more potent formulation. The combined extracts will be tested using various antioxidant assays like DPPH and ABTS and antimicrobial assays against common pathogens.

Experimental Work

Chemicals and reagents

Potassium persiflage, 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2, 2 '-azinobis-(3-ethylbenzothiazoline)- 6- sulphonic acid (ABTS), methanol, ethanol, Tris- buffer (40mM) solution, Potassium chloride (300mM) solution, Ammonium ferrous sulphate (0.16mM) solution, Ascorbic acid (0.06mM) solution Thiobarbituric acid 0.8% solution, Sodium dodecyl sulphate 8.0% solution, Acetic acid glacial 20 % solution,2M Hydrogen peroxide, Nitric acid(2M),Sodium nitrite(2M),Pyrogallol red(100µm), Berberine and gallic acid were bought from yucca enterprises, Mumbai. All other used chemicals and solvents were of analytical grade. UV visible spectrophotometer was used for the study.

Preparation of Combination of Extracts:

Among the four extracts (aqueous, methanol, chloroform, hexane) of *Apple peel* and *Golden seal root* aqueous and methanol extracts were most effective as antioxidants and antimicrobials. However, *Apple peel* extracts were superior in antimicrobial tests compared to *Golden seal root* extract. Two combinations, aqueous and methanol extracts in 1:1 ratio, were prepared for further antioxidant and antimicrobial activity testing and gel formulation.

Antioxidant Assays of Combined Extracts:

1. **DPPH Radical Scavenging Assay:** DPPH solution reacts with antioxidants, reducing its absorbance at 517 nm. Test extracts were prepared, and absorbance was measured after 30 minutes.
2. **ABTS Assay:** ABTS reacts with antioxidants, causing a decrease in absorbance at 734 nm. The intensity of the green colour produced is reduced by antioxidants.
3. **Lipid Peroxidation Inhibition Assay:** Malondialdehyde (MDA), formed by fatty acid breakdown, was measured to determine the extent of lipid peroxidation, with results analysed spectrophotometrically at 535 nm.
4. **Effect on Pyrogallol Red Bleaching by Peroxynitrite:** Peroxynitrite reacts with combined extracts, reducing the absorbance of Pyrogallol red at 540 nm.

Antimicrobial Assays of Combined Extracts:

1. **Test Organism Selection:** Gram-positive and gram-negative bacteria (*B. subtilis*, *E. coli*, *S. typhi*, *S. aureus*) were chosen for the antimicrobial study.
2. **Sample Preparation:** 1g of extract was dissolved in solvents like water, methanol, chloroform, and n-hexane. After 24 hours, filtrates were dried, dissolved in DMSO, and diluted for further tests.
3. **Test Sample Preparation:** Extract concentrations ranging from 20 to 100 mg/mL were prepared in DMSO for testing.
4. **Disc Diffusion Method:** The antimicrobial activity was tested using the disc diffusion method, measuring inhibition zones after incubating plates with selected organisms for 24 hours.

Antioxidant Assays of various combined extracts.

Scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of combined extracts.

Tab.No.1: Scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of methanol combined extracts.

Concentr		200	400	600	800	100
ABS	I	0.87	0.7	0.5	0.3	0.1
	I	0.81	0.7	0.5	0.3	0.1
	I	0.81	0.7	0.5	0.3	0.1
(Control-test)/c	I	0.08	0.2	0.4	0.6	0.7
	I	0.14	0.2	0.4	0.6	0.7
	I	0.14	0.2	0.4	0.6	0.7
Mean		0.12	0.2	0.4	0.6	0.7
Concentr		200	400	600	800	100
%INH		13.1	26.	46.	67.	80.
Std		0.03	0.0	0.0	0.0	0.0
RSD		29.9	3.7	3.3	0.8	0.1

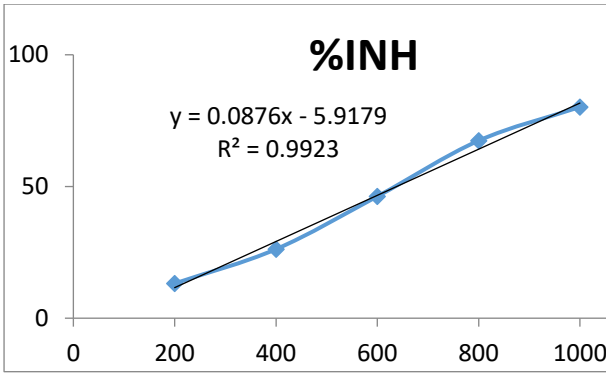

Fig. No.1: Linearity graph of Scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of methanol combined extracts.

Tab.No.2: Scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of aqueous combined extracts.

Concentrat		200	400	600	800	100
ABS	I	0.9	0.8	0.7	0.5	0.4
	I	0.9	0.8	0.6	0.5	0.4
	I	0.9	0.8	0.7	0.4	0.2
(Control-test)/control	I	-	0.1	0.1	0.3	0.4
	I	-	0.0	0.2	0.3	0.4
	I I	- 0.0	0.1 008	0.2 548	0.5 508	0.6 948
Mean		-	0.0	0.2	0.4	0.5
Concentrat		200	400	600	800	100
%INH		-	10.	23.	44.	57.
Std		0.0	0.0	0.0	0.1	0.1
RSD		0.1	1.0	1.8	3.4	4.2

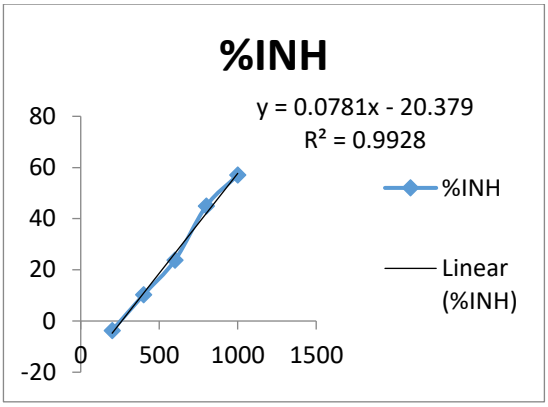

Fig.No.2: Linearity graph of scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of aqueous combined extracts ABTS Radical Scavenging Assay of two combined extracts

Tab.No.3: Scavenging Action of ABTS of methanol combined extracts

Concentra		20	400	60	80	10
ABS	I	0.6	0.4	0.3	0.2	0.1
	I	0.6	0.4	0.3	0.2	0.1
	I	0.6	0.4	0.3	0.2	0.1
(Control-test)/control	I	0.2	0.3	0.5	0.6	0.6
	I	0.2	0.4	0.5	0.5	0.7
	I I	0.2	0.3	0.5	0.6	0.7
Mean		0.2	0.3	0.5	0.6	0.7
Concentra		20	400	60	80	10
%INH		26.	44.	60.	72.	84.
Std		0.0	0.0	0.0	0.0	0.0
RSD		5.7	11.	2.5	2.9	5.0

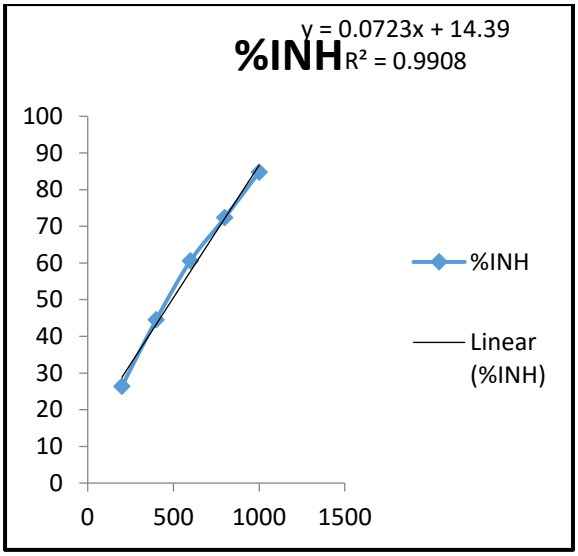

Fig.No.3 : Linearity graph of scavenging Action of ABTS of methanol combined extracts.

Tab.No.4: scavenging Action of ABTS of aqueous combined extracts.

Concentrat		200	400	600	800	100
ABS	I	1.0	0.7	0.7	0.5	0.3
	I	0.9	0.7	0.5	0.5	0.3
	I	1.0	0.7	0.7	0.5	0.3
(Control-test)/control	I	-	0.0	0.0	0.2	0.4
	I	-	0.0	0.3	0.3	0.4
	I I	- 0.1	0.0 496	0.0 486	0.2 966	0.4 596
Mean		-	0.0	0.1	0.2	0.4
Concentrat		200	400	600	800	100
%INH		-	5.7	18.	35.	55.
Std		0.0	0.0	0.1	0.0	0.0
RSD		1.3	0.0	5.0	1.0	0.4

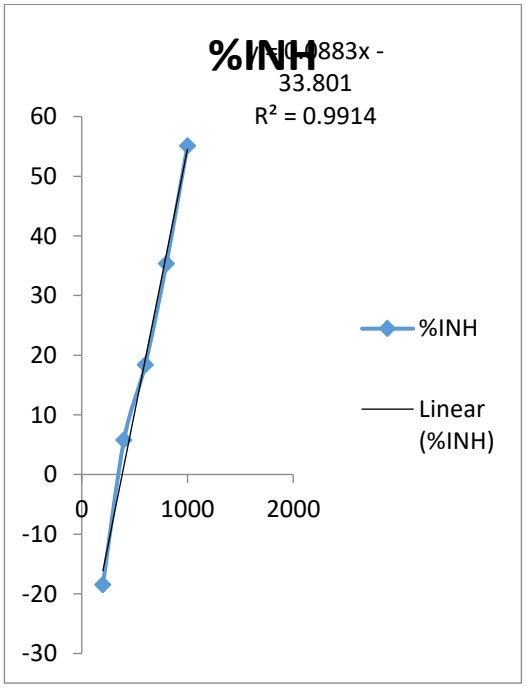

Fig.No.4: Linearity graph of scavenging Action of ABTS of aqueous combined extracts.

Effect of bleaching of Pyrogallol Red onto combined extracts by Peroxynitrite

Tab.No.5: Bleaching action of Pyrogallol red activity of methanol combined extracts

Concentra		200	40	60	80	10
ABS	I	0.9	0.7	0.6	0.4	0.1
	I	0.8	0.6	0.4	0.3	0.1
	I	0.9	0.6	0.5	0.3	0.1
(Contr ol-test)/c ontrol	I	0.0	0.2	0.2	0.5	0.8
	I	0.1	0.3	0.5	0.6	0.8
	I I	- 0.0	0.3 43	0.4 55	0.6 61	0.8 00
Mean		0.0	0.2	0.4	0.6	0.8
Concentra		200	40	60	80	10
%INH		3.5	29.	43.	64.	83.
Std		0.0	0.0	0.1	0.0	0.0
RSD		214	24.	30.	9.7	1.3

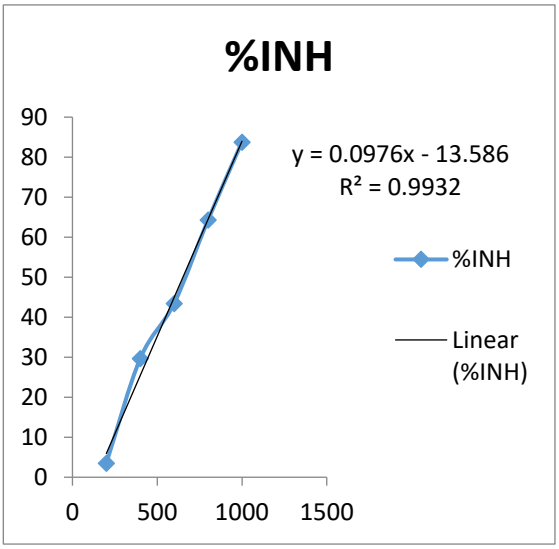

Fig.No.5: Linearity graph of bleaching action of Pyrogallol red activity of methanol combined extracts

Tab.No.6: Bleaching action of Pyrogallol red activity of aqueous combined extract

Concentrat		200	400	600	800	100
ABS	I	0.9	0.8	0.6	0.5	0.4
	I	0.9	0.6	0.5	0.5	0.4
	I	0.9	0.7	0.5	0.5	0.4
(Contr ol-test)/c ontrol	I	-	0.0	0.3	0.4	0.4
	I	-	0.2	0.3	0.4	0.5
	I I	- 0.0	0.1 789	0.4 059	0.4 019	0.4 739
Mean		-	0.1	0.3	0.4	0.4
Concentrat		200	400	600	800	100
%INH		-	18.	39.	42.	50.
Std		0.0	0.0	0.0	0.0	0.0
RSD		0.1	3.2	0.6	0.7	0.9

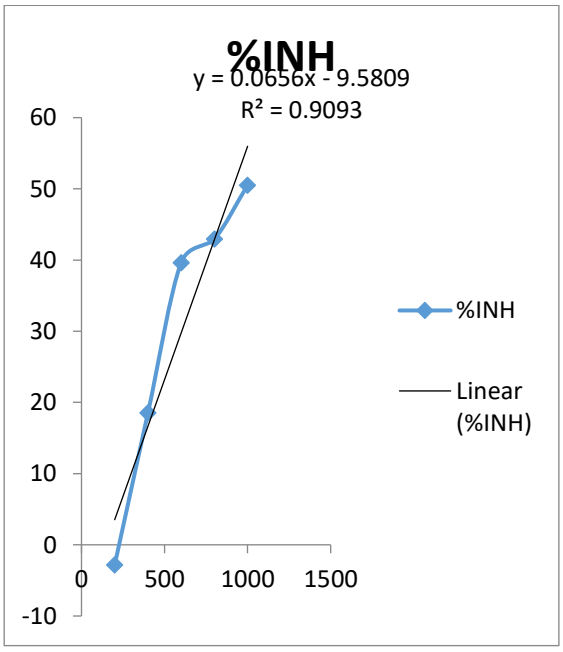

Fig.No.6: Linearity graph of bleaching action of Pyrogallol red activity of aqueous combined extract

Inhibition of Lipid Peroxidation of two combined extracts

Tab.No.7: Inhibition action of lipid peroxidation activity of methanolic combined extract

Concentration		200	400	600	800	1000
ABS	I	0.889	0.749	0.545	0.321	0.381
	I	0.883	0.749	0.545	0.450	0.219
	I	0.899	0.76	0.530	0.418	0.225
(Control-test)/control Contro	I	0.1066	0.2466	0.4506	0.6746	0.6146
	I	0.1136	0.2466	0.4506	0.5266	0.7766
	I	0.0966	0.2356	0.4666	0.5776	0.7606
Mean		0.1056	0.2429	0.4559	0.5963	0.7173
Concentration		200	400	600	800	1000
%INH		10.607	24.491	45.795	59.89	72.044
Std		0.0085	0.0064	0.0093	0.0709	0.0923
RSD		8.091	2.614	2.036	11.885	12.446

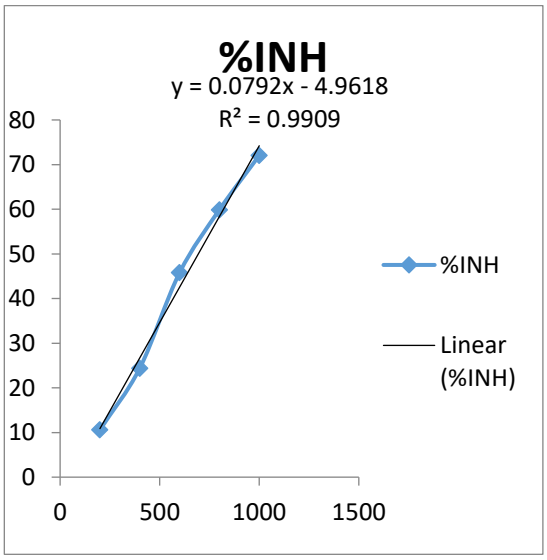

Fig.No.7: Linearity graph of Inhibition action of lipid peroxidation activity of methanol combined extract

Tab.No.8: Inhibition action of lipid peroxidation activity of aqueous combined extract

Concentrat		200	400	600	800	100
ABS	I	0.8	0.6	0.5	0.5	0.2
	I	0.8	0.8	0.5	0.4	0.2
	I	0.8	0.7	0.5	0.4	0.5
(Control-test)/control	I	0.1	0.3	0.4	0.4	0.6
	I	0.1	0.0	0.4	0.5	0.7
	I I	0.1 056	0.2 006	0.3 966	0.5 166	0.4 266
Mean		0.1	0.2	0.4	0.4	0.6
Concentrat		200	400	600	800	100
%INH		10.	22.	40.	48.	61.
Std		0.0	0.1	0.0	0.0	0.1
RSD		0.0	4.8	0.1	2.2	5.3

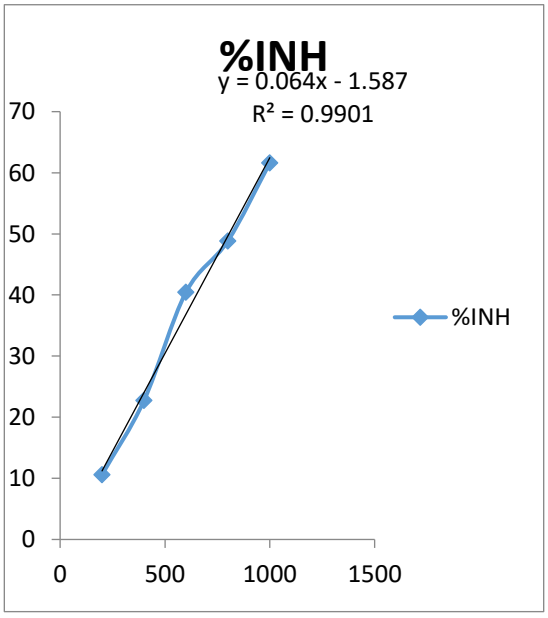

Fig.No.8: Linearity graph of Inhibition action of lipid peroxidation activity of aqueous combined extracts

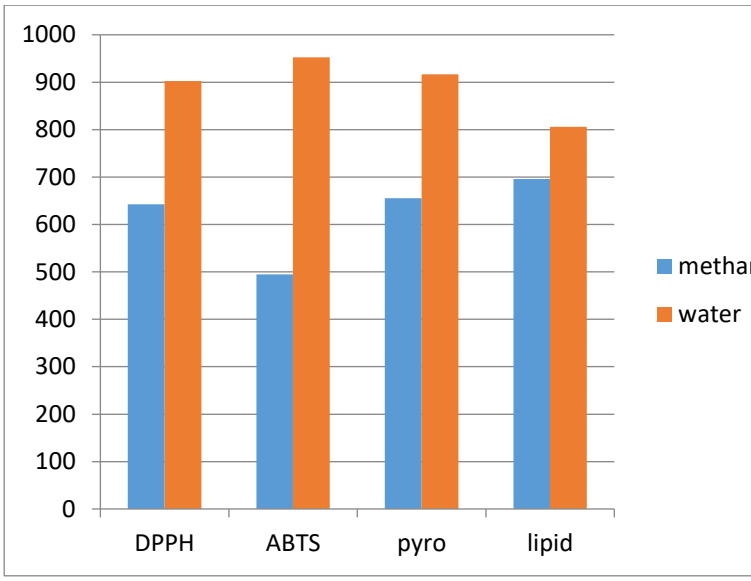

Fig No.9: Comparison of IC50 values of combined extract

Results and Discussion of Antioxidant and Antimicrobial Activity of Combined Extracts

In-vitro Antioxidant Assay of Combined Extract

1. **Scavenging Action of 2,2-Diphenyl-1-Picrylhydrazyl (DPPH):**
   The DPPH assay measures the antioxidant activity of a sample by its ability to donate hydrogen atoms to neutralize free radicals. When the combined extract acts as an antioxidant, it reduces the purple DPPH solution to a yellow compound, diphenyl picrylhydrazine. The decrease in absorbance at 517 nm indicates

increased antioxidant activity. The alcoholic combined extract showed significant DPPH radical scavenging ability with an IC50 value of 642.72 µg/ml, which is comparable to the reference Apple peel (IC50 = 635 µg/ml).

2. **ABTS Radical Scavenging Assay:** In the ABTS assay, the combined extract reacts with the ABTS+• radical cation, which has a bluish-green color, formed by the reaction of ABTS with potassium persulfate. Antioxidants reduce the color intensity by scavenging the ABTS+• radicals. The IC50 value of the combined extract for scavenging 50% of the ABTS+• radicals was 494.98 µg/ml, demonstrating its strong antioxidant capacity. Ascorbic acid was used as the reference standard for comparison, and each test was repeated three times for statistical accuracy.
3. **Bleaching Action of Pyrogallol Red by Peroxynitrite:** In this assay, peroxynitrite radicals bleach the dark red color of pyrogallol red. Antioxidants protect the dye from decolorization by neutralizing the peroxynitrite radicals, thus retaining the color intensity. The combined extract showed significant inhibition of pyrogallol red bleaching with an IC50 value of 655.46 µg/ml, compared to the standard ascorbic acid.

Conclusion:

The combined extracts demonstrated potent antioxidant activity in all assays, with effective scavenging of DPPH, ABTS+•, and peroxynitrite radicals. The alcoholic combined extract, in particular, showed strong performance across the tests, indicating its potential as a natural antioxidant agent. These findings support the further exploration of the combined extracts for antioxidant applications in both pharmaceutical and cosmetic formulations.

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