International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 8, August, 2025
Page No.: 13-21

Research

Evaluation of Synergistic Potential of Combined Extracts of Apple Peel and Goldenseal Root Extract

Pratiksha P. Nikam[1], Dr. Sanjay K. Bais[2], Dr. Javesh K Patil[3]

[1]Ph.D Research Scholar, JJTU Jhunjhunu Rajasthan.

[2,3]Professor JJTU Jhunjhunu Rajasthan.

Corresponding Author:
Pratiksha P. Nikam
Email: *NA*

DOI: *10.62896/ijpdd.2.8.02*

Conflict of interest: *NIL*

Article History
Received: 12/07/2025
Accepted: 25/08/2025
Published: 30/08/2025

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.

Keywords: *apple peel and goldenseal root extracts, DPPH radical,*

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.[1-3]

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.[4]

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 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.

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 8, August, 2025
Page No.: 13-21

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

Concentration		200	400	600	800	1000
ABS	I	0.877	0.719	0.519	0.319	0.191
	II	0.812	0.705	0.529	0.309	0.191
	III	0.811	0.701	0.5	0.311	0.189
(Control-test)/control Control=0.95	I	0.0828	0.2408	0.4408	0.6408	0.7688
	II	0.1478	0.2548	0.4308	0.6508	0.7688
	III	0.1488	0.2588	0.4598	0.6488	0.7708
Mean		0.126467	0.25147	0.4438	0.6468	0.76947
Concentration		200	400	600	800	1000
%INH		13.17636	26.1999	46.2388	67.389	80.1695
Std		0.03782	0.00945	0.01473	0.00529	0.00116
RSD		29.9050	3.7587	3.3193	0.8182	0.1501

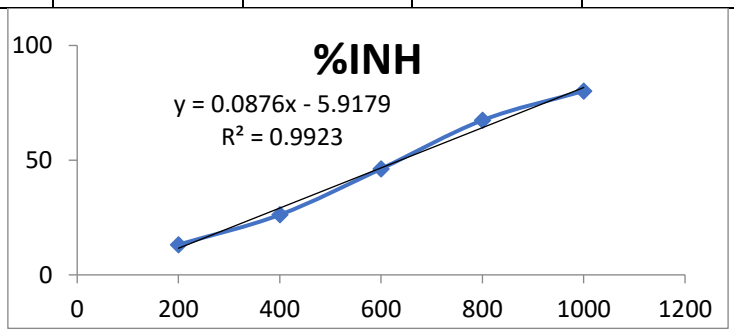

Fig. No.1: Linearity graph of Scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of methanol combined extracts.

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

Concentration		200	400	600	800	1000
ABS	I	0.99	0.83	0.795	0.598	0.495
	II	0.997	0.895	0.695	0.577	0.478
	III	0.999	0.859	0.705	0.409	0.265
(Control-test)/control Control=0.95	I	-0.03	0.1298	0.1648	0.3618	0.4648
	II	-0.037	0.0648	0.2648	0.3828	0.4818
	III	-0.039	0.1008	0.2548	0.5508	0.6948
Mean		-0.036	0.0985	0.2281	0.4318	0.5471
Concentration		200	400	600	800	1000
%INH		-3.702	10.259	23.769	44.989	57.005

Std	0.0047	0.0326	0.0551	0.1036	0.1282
RSD	0.1575	1.0854	1.8359	3.453	4.2722

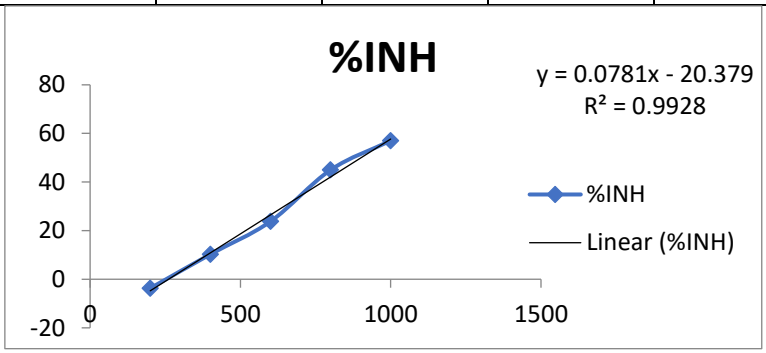

Fig.No.2: Linearity graph of scavenging Action of 2, 2-Diphenyl-1-Picrylhydrazyl of aqueous combined extracts

ABTS Radical Scavenging Assay of two combined extracts

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

Concentration		200	400	600	800	1000
ABS	I	0.629	0.49	0.339	0.223	0.171
	II	0.633	0.421	0.343	0.255	0.109
	III	0.609	0.499	0.319	0.225	0.107
(Control-test)/control Control=0.84	I	0.2186	0.3576	0.5086	0.6246	0.6766
	II	0.2146	0.4266	0.5046	0.5926	0.7386
	III	0.2386	0.3486	0.5286	0.6226	0.7406
Mean		0.2239	0.3776	0.5139	0.6133	0.7186
Concentration		200	400	600	800	1000
%INH		26.42	44.5493	60.634	72.353	84.781
Std		0.0129	0.04267	0.0129	0.0179	0.0364
RSD		5.7429	11.3003	2.5023	2.9237	5.0640

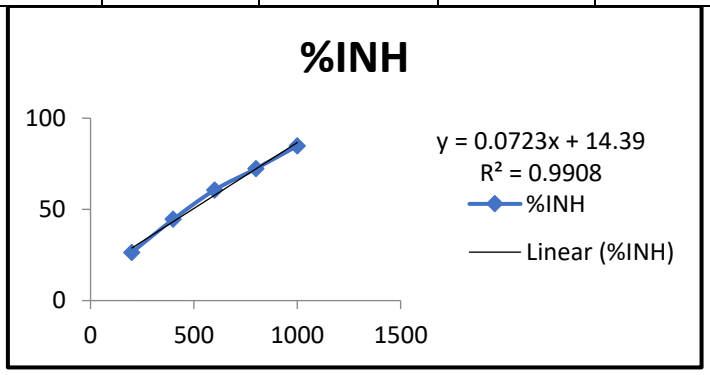

Fig.No.3: Linearity graph of scavenging Action of ABTS of methanol combined extracts.

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 8, August, 2025
Page No.: 13-21

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

Concentration		200	400	600	800	1000
ABS	I	1.04	0.799	0.76	0.579	0.389
	II	0.959	0.799	0.517	0.515	0.365
	III	1.014	0.798	0.799	0.551	0.388
(Control-test)/control Control=0.84	I	-0.192	0.0486	0.0876	0.2686	0.4586
	II	-0.111	0.0486	0.3306	0.3326	0.4826
	III	-0.166	0.0496	0.0486	0.2966	0.4596
Mean		-0.157	0.0489	0.1556	0.2993	0.4669
Concentration		200	400	600	800	1000
%INH		-18.49	5.7732	18.358	35.308	55.089
Std		0.0414	0.0006	0.1528	0.0321	0.0136
RSD		1.3785	0.0193	5.0935	1.0694	0.4526

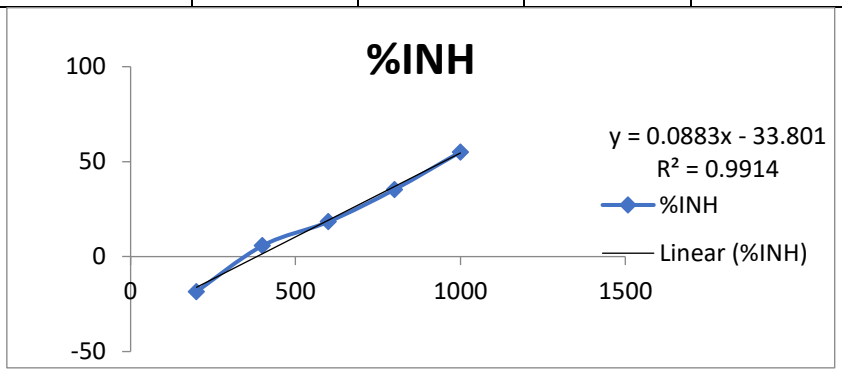

Fig.No.4: Linearity graph of scavenging Action of ABTS of aqueous combined

Effect of bleaching of Pyrogallol Red onto combined extracts by Peroxynitrite

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

Concentration		200	400	600	800	1000
ABS	I	0.959	0.759	0.691	0.415	0.159
	II	0.851	0.659	0.439	0.315	0.145
	III	0.991	0.624	0.512	0.306	0.167
(Control-test)/control Control=0.96	I	0.0089	0.2089	0.2769	0.5529	0.8089
	II	0.1169	0.3089	0.5289	0.6529	0.8229

	III	-0.0231	0.3439	0.4559	0.6619	0.8009
Mean		0.0342	0.2872	0.4206	0.6226	0.8109
Concentration		200	400	600	800	1000
%INH		3.5369	29.676	43.452	64.321	83.779
Std		0.0734	0.0701	0.1297	0.0605	0.0111
RSD		214.315	24.392	30.830	9.718	1.374

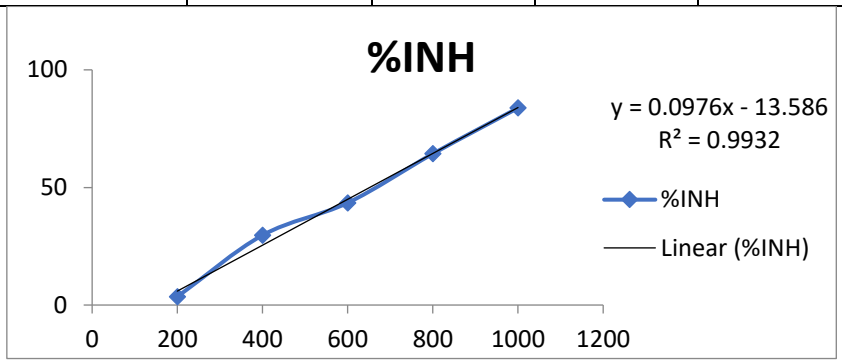

Fig.No.5: Linearity graph of bleaching action of Pyrogallol red activity of methanol combined extracts

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

Concentration		200	400	600	800	1000
ABS	I	0.998	0.886	0.6	0.566	0.498
	II	0.989	0.691	0.591	0.525	0.445
	III	0.999	0.789	0.562	0.566	0.494
(Control-test)/control Control=0.96	I	-0.03	0.0819	0.3679	0.4019	0.4699
	II	-0.021	0.2769	0.3769	0.4429	0.5229
	III	-0.031	0.1789	0.4059	0.4019	0.4739
Mean		-0.027	0.1792	0.3836	0.4156	0.4889
Concentration		200	400	600	800	1000
%INH		-2.834	18.518	39.629	42.935	50.511
Std		0.0055	0.0975	0.0199	0.0237	0.0295
RSD		0.1836	3.25	0.6619	0.789	0.9838

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 8, August, 2025
Page No.: 13-21

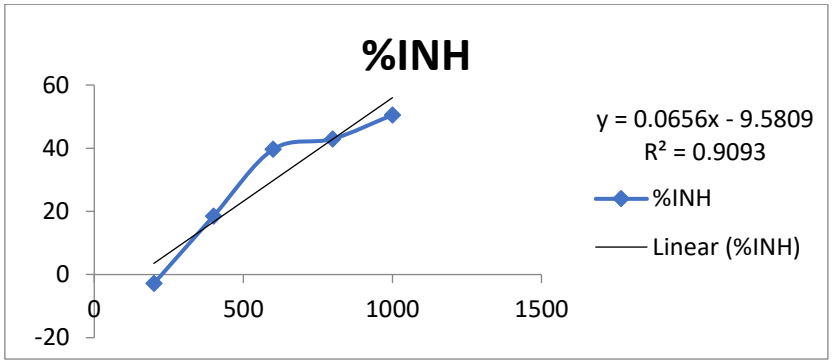

Fig.No.6: Linearity graph of bleaching action of Pyrogallol red activity of aqueous combined extract

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
	II	0.882	0.749	0.545	0.459	0.219
	III	0.899	0.76	0.529	0.418	0.235
(Control-test)/control Control=0.99	I	0.1066	0.2466	0.4506	0.6746	0.6146
	II	0.1136	0.2466	0.4506	0.5366	0.7766
	III	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	24.401	45.795	59.89	72.044
Std		0.0085	0.0064	0.0092	0.0709	0.0893
RSD		8.091	2.614	2.026	11.885	12.446

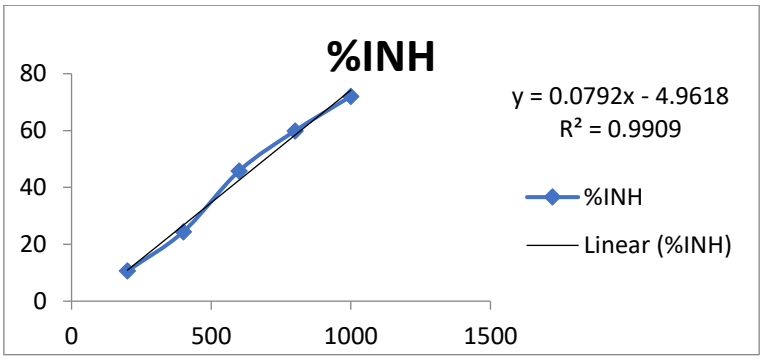

Fig.No.7: Linearity graph of Inhibition action of lipid peroxidation activity of methanol combined extract

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

Concentration		200	400	600	800	1000
ABS	I	0.891	0.614	0.589	0.588	0.299
	II	0.889	0.899	0.591	0.461	0.279
	III	0.89	0.795	0.599	0.479	0.569
(Control-test)/control Control=0.99	I	0.1046	0.3816	0.4066	0.4076	0.6966
	II	0.1066	0.0966	0.4046	0.5346	0.7166
	III	0.1056	0.2006	0.3966	0.5166	0.4266
Mean		0.1056	0.2263	0.4026	0.4863	0.6133
Concentration		200	400	600	800	1000
%INH		10.607	22.727	40.438	48.842	61.598

Std	0.001	0.1442	0.0053	0.0687	0.162
RSD	0.0333	4.8074	0.1764	2.2906	5.3989

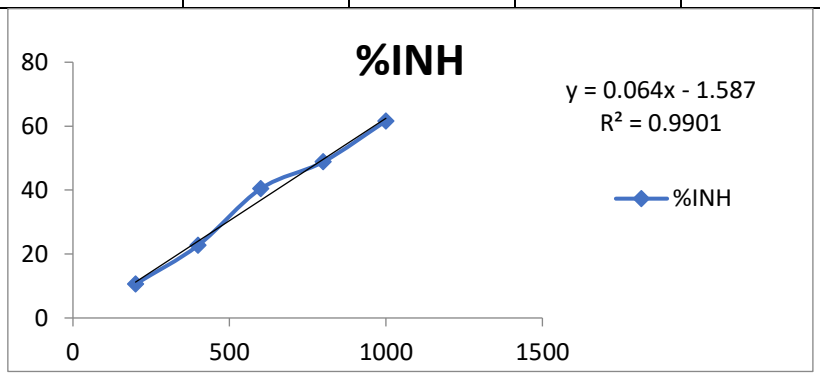

Fig.No.8: Linearity graph of Inhibition action of lipid peroxidation activity of aqueous combined extracts

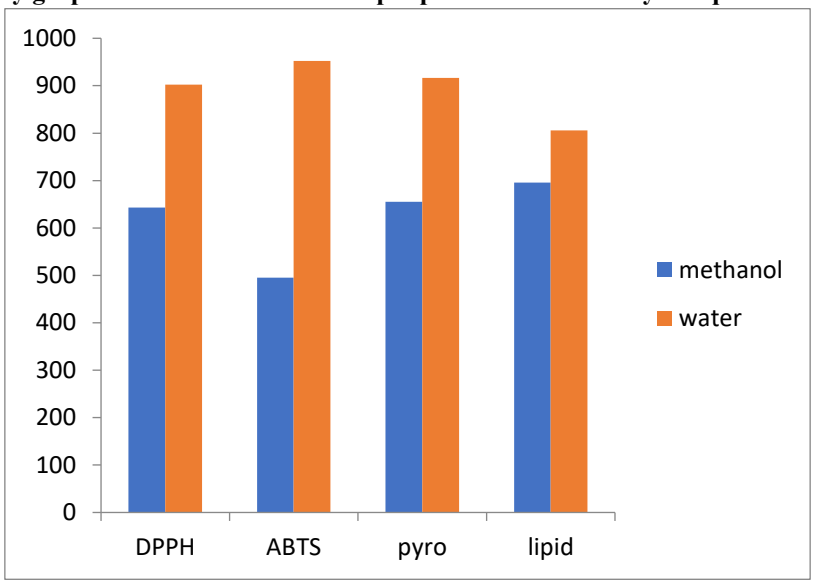

Fig No.9: Comparison of IC50 values of combined extract

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.

REFERENCES

1. *Munasinghe TC, Seneviratne CK, Thabrew MI, Abeysekera AM. Antiradical and anti-lipoperoxidative effects of some plant extracts used by Sri Lankan traditional medical practitioners for cardio-protection. Phytother Res 2001;15:519–23.*
2. *Miliauskas G, Venskutonis PR, Van BeekTA.Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 2004;85:231–237.*
3. *Deepali CM, Usha SS, Pratima AT, Vikrama N. Evaluation of Punicagranatum fruit peels extracts for its free radical scavenging and anti-inflammatory activity. Int J PharmPharmSci 2015;7:222-225.*
4. *Boonchum W, Peerapornpisal Y, Kanjanapothi D. Antioxidant activity of some seaweed from the Gulf of Thailand. IJAB 2011;13:95–99.*
5. *Mohan PS, Becker K. Studies on antioxidant activity of Indian (Cassia fistula linn) a preliminary assessment of crude extract from stem, flower, bark. Food chem 2002;79:61-67.*
6. *K. Basappa, J. VenuGopal. Natural Alternatives to Antibiotic Agents. Asian Journal of Biomedical and Pharmaceutical Sciences 2013;3:1-4.*
7. *Bhardwaj M, Singh BR, Sinha DK, Kumar V, PrasannaVadhana OR, Varan Singh S, Nirupama KR, Pruthvishree and ArchanaSaraf BS. Potential of Herbal Drug and Antibiotic Combination Therapy: A New Approach to Treat Multidrug Resistant Bacteria.Pharm Anal Acta2016;7:11*
8. *Yamauchi A. Gels: Introduction. In: Osade Y, Kajiwara K, editors. Gels Handbook. San Diego: The Fundamentals, Academic Press 2001;1:4- 12.*
9. *Jurenka JS. Therapeutic applications of pomegranate (Punicagranatum L.): a review. Altern Med Rev 2008;13:128-44.*
10. *Dudonné S, Vitrac X, Coutiére P, Woillez M, MérillonJM.Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. J AgriFoodChem 2009;57:1768–1774.*
11. *SachinSS, RchanaRJ,Manoj NG. In-vitro antioxidant and anti-inflammatory activity of methanol extract of Oxalis corniculatalinn. Int. J. Pharm pharmsci 2010;2:146-155.*
12. *Saha A, Ahmed M. The analgesic and anti-inflammatory activities of the extracts of Albizialebbeckin animal model.Pak.J.Pharm.Sci2009;22(1):74-77.*
13. *Dorle AD, Swami KS, Nagare SK, Hyam SR. Design and evaluation of novel topical gel of tinospora cordifolia as antimicrobial agent. Asian J Pharm Clin Res 2015; 6:237-239. 11.*
14. *Dwivedi S, Gupta S. Formulation and evaluation of herbal gel containing sesbaniagrandiflora (l.) poir. leaf extract. ActaChim Pharm Indica 2012;2:54-59.*
15. *Goyal S, Sharma P, Ramchandani U, Shrivastava SK, Dubey PK. Novel Anti-inflammatory topical herbal gels containing withaniasomnifera and boswelliaserrata. IJPBA 2011; 2:1087-1094.*
16. *Ozgen, VO and Oyetayo, FL.Phytochemical Screening and antibacterial Properties of siam weed, chromolaerzaodrata leaf against aerobic isolated of Wound. J Applied Environ Sci 2006;2:7-11*
17. *Murugan, T. Antimicrobial activity of leaves and latex extract of the herbal plant calatropisgigantea; IJBPAS 2012;1: 261-270*

International Journal of Pharmaceutical Drug Design (IJPDD)
Website: https://ijpdd.org/
ISSN: 2584-2897
Vol. 2, Issue 8, August, 2025
Page No.: 13-21

*****