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ANTIOXIDANT ACTIVITY ASSESSMENT OF BINARY POLYHERBAL FORMULATIONS CONTAINING HYDROETHANOLIC EXTRACTS OF CURCUMA LONGA AND TERMINALIA ARJUNA

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ABSTRACT Medicinal plants such as Curcuma longa and terminalia arjuna have been widely used as traditional medicines for the treatment of chronic diseases. The present study aimed to extract the ultrasound-assisted hydroalcoholic extracts of C. longa and T. arjuna for their phytochemical composition, antioxidant activity, and their interactions in binary formulations. Preliminary phytochemical screening confirmed the presence of phenolics, flavonoids, tannins, glycosides, and terpenoids in both extracts. T. arjuna showed higher total phenolic (220.99±4.495 mg GAE/g) and flavonoid content (121.78±1.984 mg QE/g) than C. longa (117.42±2.696 mg GAE/g and 86.89±1.424 mg QE/g, respectively). Antioxidant activity was assessed using DPPH radical and nitric oxide scavenging assays (NOSA). Among the individual extracts, T. arjuna (90.73±0.495 μg/mL) showed stronger DPPH scavenging activity, whereas C. longa (139.8±1.723 μg/mL) exhibited comparatively better nitric oxide scavenging activity. Among the binary formulations, F2 (equal proportions of C. longa and T. arjuna extracts) resulted the strongest antioxidant activity with IC50 values of 82.77±0.42 μg/mL (DPPH) and 106.9±4.003 μg/mL (NOSA). The Chou–Talalay Combination Index (CI) method confirmed and quantified the synergistic interaction of F2. The findings suggest that synergistic evaluation of binary polyherbal formulation F2 using the CI method provides an effective approach for the development of novel natural antioxidant formulations.

KEYWORDS : Phytochemicals, Antioxidant Potential, Polyherbal Formulation, Synergism

INTRODUCTION

Antioxidants can neutralize or scavenge the free radicals generated during body's cellular metabolism. However excessive generation of free radicals surpasses the capacity of body's endogenous antioxidant defence systems, leading to oxidative stress related cellular damage and progression of chronic disorders such as inflammatory, cardiovascular diseases, diabetes, cancer, neurodegenerative diseases[1]. Medicinal plants are rich sources of bioactive phytochemicals including polyphenols, flavonoids, glycosides, and terpenoids possessing diverse pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, and anticancer activities[2]. According to the World Health Organization (WHO), nearly 80% of the global population relies on herbal medicines for primary healthcare and as an alternative to synthetic antioxidants[2,3].

Traditional medicinal systems predominantly employ polyherbal formulations, where combinations of medicinal plants are believed to enhance therapeutic efficacy through synergistic phytochemical interactions. Such combinations may improve biological activity by targeting multiple pathways while reducing the higher doses associated with single-herb therapies[3].

Curcuma longa L. (Zingiberaceae), commonly known as turmeric, has been extensively used in traditional medicine for the management of inflammation conditions, and wound healing. Its major bioactive constituents are curcumininoid and other consitituents like Turmerones, α-Curcumene, reported to possess significant antioxidant, antimicrobial and anti-inflammatory activities[4]. Terminalia arjuna Roxb. (Combretaceae) is traditionally used in the treatment of cardiovascular disorders and bleeding conditions. The bark contains phytochemicals including Arjunin, Arjunic acid, and Gallic acid contributing to its strong antioxidant and cardioprotective properties[5]. However, limited studies have explored the antioxidant potential of binary hydroethanolic extract combinations of Curcuma longa and Terminalia arjuna. Therefore, the present study was aimed to prepare a polyherbal binary combinations of C. longa and T. arjuna and evaluate their antioxidant activity and the synergistic interactions using the Combination Index (CI) approach.

MATERIALS AND METHODS

Plant Material Collection and Authentication

The rhizomes of Curcuma longa and raw bark of Terminalia arjuna was collected from the tree in the month of June 2025 from Somaiya Vidyavihar campus, Mumbai (Maharashtra), India. Plant specimens were authenticated at the Blatter Herbarium, St. Xavier's College, Mumbai, and were identified as Curcuma longa L matching with specimen no. B.R.-470 of Rukmini Bai and Terminalia arjuna (Roxb. ex DC.) Wight & Arn. matching with NYD-2744 of N. Y. Das. The collected material was washed, shade-dried, powdered using a domestic electric grinder and used for further experiments.

Extraction of Plant Material

The powdered plant materials were subjected to ultrasound-assisted extraction using hydroethanolic solvent (ethanol: water, 7:3 v/v) in a 1:10 ratio of plant material to solvent. Extraction was carried out in a sonication bath for 45 min. Extracts were filtered and concentrated under vacuum. Dried hydroethanolic extracts of Curcuma longa (CL) and Terminalia arjuna (TA) were mixed in different ratios (F1(25:75), F2(50:50), F3(75:25) of C. longa:T. arjuna) to prepare binary polyherbal formulations.

Determination of Total Phenolic and Flavonoid Content

Total phenolic content in the extracts was determined by Folin-Ciocalteu method[7] and absorbance was measured at 765 nm. Gallic acid was used as standard, and results were expressed as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g). Total Flavonoid content in the extracts was determined by aluminium chloride method[7] and Absorbance was measured at 510 nm. Quercetin was used as standard, and results were expressed as milligrams of quercetin equivalent per gram of dry extract (mg QE/g).

Antioxidant Activity:

The antioxidant capacity of the extracts or formulations was evaluated using 2,2-Diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH-RSA) and nitric oxide scavenging assay (NOSA)[8]. For the DPPH-RSA assay, aliquots of 0.1 mM DPPH solution were mixed with extracts or combinations and incubated in dark for 30 min at room temperature, and absorbance was measured at 517 nm. For NOSA, sodium

nitroprusside was used as for Nitric oxide generation and the absorbance was recorded at 546 nm. Ascorbic acid was used as reference standard, and percentage inhibition was calculated using the following equation:

$$\% \text{ Scavenging} = \frac{A_0 - A_s}{A_0} \times 100$$

where A_0 is the absorbance of control and A_s is the absorbance of sample.

Synergy Assessment of Binary Formulations

Interactions among the binary formulations were evaluated using the Chou–Talalay Combination Index (CI) method in terms of its antioxidant activity[9]. The synergistic interaction between the extracts in formulation was determined according to the following equation:

$$CI = \frac{d_1}{D_1} + \frac{d_2}{D_2}$$

Where D_1 and D_2 is the IC_{50} values of individual extracts, required to produce specific effect; d_1 and d_2 is the IC_{50} values of individual constituent in the combination required to produce the same effect respectively.

RESULT AND DISCUSSION

The phenolic and flavonoid compounds are the major phytoconstituents responsible for the antioxidant activity due to their ability to act as electron donors, hydrogen donors, and metal ion chelators[2]. Among the extracts, T. arjuna exhibited the highest total phenolic content (220.99 ± 4.495 mg GAE/g) and total flavonoid content (121.78 ± 1.984 mg QE/g), compared with C. longa, which showed 117.42 ± 2.696 mg GAE/g and 86.89 ± 1.424 mg QE/g, respectively. The high phenolic and flavonoid content in the extract indicates the efficient extraction technique, which enhances solvent penetration and facilitates the release of bioactive phytoconstituents from plant matrices[10].

Antioxidant Activity

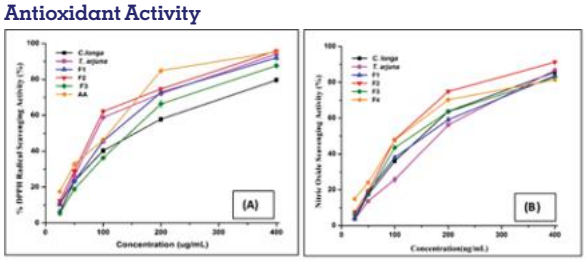

Fig.1 % Scavenging profiles of individual and binary formulations of *Curcuma longa* and *Terminalia arjuna* of hydroethanolic extracts. (A) DPPH radical scavenging activity and (B) Nitric oxide scavenging assay

The Scavenging profiles of the individual extracts and all binary formulations exhibited increasing concentration-dependent free radical scavenging activity (Fig. 1 (A) and (B)). Multiple assay techniques were employed to get the equivocal results, and their potential was compared using Inhibitory Concentration (IC_{50}, concentration required inhibit 50% of the process) values where, low IC_{50} value indicates higher antioxidant activity (Table 1). In the DPPH assay, T. arjuna showed stronger scavenging activity than C. longa, while among the formulations, F2 (50:50) exhibited the highest antioxidant potential with the lowest IC_{50} value. Similarly, in the nitric oxide scavenging assay, F2 demonstrated the strongest activity followed by F3 and F1, whereas C. longa showed comparatively better activity than T. arjuna. Variations in antioxidant activity among the previous literatures may also be influenced by differences in phytochemical composition, extraction methods, solvent polarity, and geographical factors.

Synergy Assessment of Binary Formulation

The synergistic interactions among the binary formulations were evaluated using the Chou–Talalay combination index (CI) method based on the IC_{50}values obtained from DPPH and nitric oxide scavenging assays (Table1). In the DPPH-RSA assay, F2 exhibited synergistic interaction with a CI value of 0.70, whereas F1 and F3 showed near additive interactions. Similarly, in the NOSA, F2 demonstrated the strongest synergistic interaction with a CI value of 0.69, while F1 and F3 showed moderate synergism. The synergistic antioxidant interaction observed in F2 may probably cause in due to the complementary action of phytochemicals from C. longa and T. arjuna, their of reaction rates, polarity, relative proportion and concentration of the interacting Compounds[11].

Table 1. Combination Index (CI) analysis of hydroethanolic binary formulations of Curcuma longa and terminalia arjuna extracts

Assay	Formulations	Ic50 (ug/mL) (Experimental)	IC50 (ug/mL) (Predicted)	CI	Interactions
DPPH-RSA	F1	107.07 ± 1.747	103.93 ± 1.178	1.03	Additive
	F2	82.77 ± 0.42	117.13 ± 1.920	0.70	Synergism
	F3	133.1 ± 2.32	130.33 ± 2.671	1.02	Additive
	C. longa	143.53 ± 3.427	-	1	-
	T. arjuna	90.73 ± 0.495	-	1	-
	Ascorbic Acid	85.57 ± 1.421	-	-	-
NOSA	F1	144.67 ± 3.405	160.79 2.652	0.89	Moderate synergism
	F2	106.9 ± 4.003	153.82 1.521	0.69	Synergism
	F3	131.8 ± 1.534	146.841 1.176	1.88	Moderate Synergism
	C. longa	139.87 ± 1.723	-	1	-
	T. arjuna	167.77 ± 3.299	-	1	-
	Ascorbic acid	109.53 ± 3.009	-	-	-

Ratio of extracts in binary formulation. F1(25:75) :F2(50:50);F3(75:25)
CI=1(additive), CI <1(synergism), CI>1(antagonism)

CONCLUSION

The current study demonstrated that ultrasound-assisted hydroalcoholic extraction effectively enhanced the extraction of bioactive phytoconstituents from C. longa and T. arjuna. Both extracts showed higher phenolic and flavonoid content along with significant antioxidant potential helps a preliminary assessment of a polyherbal potential activity. Among the binary formulations, F2 (50:50) exhibited the strongest antioxidant capability with the lowest IC_{50} values and significant synergistic interaction in both assays. The observed activity offers an understanding of interactions among the phytochemicals when combined. This study lays the preliminary groundwork for developing a standardized turmeric–arjuna polyherbal extract for oxidative stress related disorders. Overall, the findings suggest that the binary formulation in equal proportions of C. longa and T. arjuna could can serve as natural antioxidant combination. However, further anti-inflammatory potential investigation and clinical trials are required to elucidate its efficacy.

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