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Phytochemical Synergy and Antioxidant Enzyme Activity of Azadirachta indica and Triticum aestivum Extracts

Aniqa Qasim[1], Khizir Abbas[2], Noor Ul Huda[3], Mariam Amir[4]

[1]Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan
[2]Department of Botany, University of Education Lahore, Dera Ghazi Khan Campus, Punjab, Pakistan
[3]Department of Microbiology, Biological Science, Quaid-e-Azam University, Islamabad, Pakistan
[4]Department of Biochemistry, University of Agriculture Faisalabad, Punjab, Pakistan.

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Correspondence to: Aniqa Qasim, Department of Botany, The Islamia University of Bahawalpur, Punjab, Pakistan.
Email: aniqaqasim761@gmail.com

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ABSTRACT

Rising demand for herbal products for therapeutic applications has driven the examination of synergistic effects between medicinally plants and nutraceutical plants. Azadirachta indica (Neem) is known for its numerous phytochemicals such as flavonoids, alkaloids, terpenoids and phenolics that display strong antimicrobial, anti-inflammatory and antioxidant properties. Likewise, Triticum aestivum (Wheatgrass) has been reputed for containing chlorophyll, amino acids, vitamins, and phenolic compounds, some of which involved in detoxification, immuno-modulation, and anti-oxidant activity. The current study is aimed at understanding synergistic interactions between Neem and Wheatgrass extracts in context to qualitative and quantitative phytochemical profiling and antioxidant enzyme activities. Bioactive components were determined using standard phytochemical screening methods, while quantitative determination of proteins and phenolics were done. Enzymatic anti-oxidant assays for catalase, peroxidase and superoxide dismutase were carried out to check oxidative defense capability of extracts both separately and in combination. The results showed that combined extracts possessed a higher antioxidant enzymatic activity compared to non-combined arrangements, which indicated that bioactive compounds were potentiating each other. The present investigation emphasizes the potential use of polyherbal formulations for enhancing the antioxidant defense and endorses the traditional as well as modern use of Neem and Wheatgrass for nutraceutical as well as therapeutic preparations.

INTRODUCTION

Since ancient times, plants have been used for medicinal purposes because of bioactive compounds in their composition with therapeutic effects. During the past few decades, there has been a focus in polyherbal and synergistic formulations as they are known to show superior pharmacological activity compared to the single plant extracts (Patwardhan et al., 2015). Neem and Wheatgrass are traditionally well known medicinally important plant contains various phytoconstituents and therapeutic actions.

Azadirachta indica (family: Meliaceae) is one of the most commonly used medicinal plants, known for its antimicrobial, anti-inflammatory, antidiabetic, and antioxidant effects in traditional systems. The bioactive compounds of neem like azadirachtin, nimbin, qurcetin, flavonoids and limonoids are documented for its high free radical scavenging ability (Subapriya & Nagini, 2005; Kausik et al., 2002). Neem leaves and seed extracts mediated regulation of oxidative stress markers and improved enzymatic and enzymatic antioxidant defence system of an organism have been reported (Alzohairy, 2016).

Wheatgrass (Triticum aestivum, family: Poaceae) is used globally as a nutraceutical because of its high content of chlorophyll, vitamins, amino acids, and phenolic compounds. Wheatgrass extracts show antioxidant, anti-inflammatory, and immunomodulatory properties and, therefore, can be used for health promoting supplements (Kulkarni et al., 2006; Ben-Arye et al., 2015).

Both Neem and Wheatgrass have been well studied for their phytochemical content as well as biological activities, but the synergizing characteristics have been studied to a limited extent. Phytochemical synergy could be a new promising avenue, in which bioactive ingredients of two or more plants act synergistically, thereby supporting a

higher overall pharmacological effect (Wagner, 2011). Knowledge about such synergistic interactions is necessary for a rational design of efficient polyherbal formulations.

Accordingly, the current study was aimed at evaluating the synergistic phytochemical composition and antioxidant enzyme activity of Neem and Wheatgrass extracts. The objective of the present investigation is to assess the benefits of Neem, Wheat synergy in augmenting the antioxidant defence responses of the system by employing qualitative and quantitative phytochemical screening and catalase, peroxidase and superoxide dismutase assays.

Problem Statement

Although number of studies have explained the phyto-constituents and antioxidant activity of Azadirachta indica (Neem) and Triticum aestivum (Wheatgrass) individually, but there is a lack of studies on its synergistic approach. Because of the fact that phytochemicals generally additively or synergistically act to increase biological activity, the lack of such research with combined extracts would be a major gap of knowledge. The study of the synergistic effects of Neem and Wheatgrass on antioxidant enzymes thus appears crucial to validate its use in nutraceutical and therapeutic formulations.

Research Questions

1. What are the phytochemical contents of the extracts from Azadirachta indica (Neem) and Triticum aestivum (Wheatgrass) separately?
2. What influence the qualitative and quantitative phytochemical profile of the combined extract of Neem and Wheatgrass as compared with the individual extract?
3. Do the synergistic extracts show increased antioxidant enzyme activities (catalase, peroxidase and superoxide dismutase) in comparison with the only extracts?
4. Is the synergistic antioxidant activity visualized help in proving that Neem & Wheat formulations could be a prospect for nutraceutical and therapeutic purpose?

Research Objectives

- To carry out qualitative phytochemical profiling of Azadirachta indica (Neem) & Triticum aestivum (Wheatgrass) extracts single & combined.
- To determine the levels of major groups of bioactive compounds i.e. total phenolics and proteins, in individual and synergy extracts.
- To determine the activities of antioxidant enzymes (catalase, rooted in Neem, Wheatgrass and their combination extracts.
- To evaluate if the synergistic mixture has a semulative effect in relation to the antioxidant potential of the single extracts.
- To rationalize the use of Neem, Wheat synergy in Nutraceutical, and Therapeutic formulations.

LITERATURE REVIEW

Azadirachta Indica (Neem)

Neem is known to be a versatile medicinal plant, containing a number of phytochemicals such as limonoids (azadirachtin, azadiradione), flavonoids, tannins, and phenolic compounds (Subapriya & Nagini, 2005). These constituents are responsible for its antimicrobial, antidiabetic, hepatoprotective, and antioxidants properties (Kausik et al., 2002). Neem extracts are reported to decrease oxidative stress as well as to modulate antioxidant enzymes, thus prompting endogenous defence systems (Alzohairy, 2016). Hence, azadiradione has also been found as superoxide dismutase (SOD) mimicking, directly quenching superoxide radicals (Verma et al., 2020). Such results confirm the antioxidative property of neem.

Triticum Aestivum (Wheatgrass)

Wheatgrass (Triticum aestivum) is a nutraceutical descended from medicinal chlorophyll, amino acids, vitamins and minerals and phenolic compounds. It is known to possess antioxidant (Kulkarni et al., 2006), anti-inflammatory, and immunomodulatory effects. Wheatgrass supplementation: A source of natural antioxidants, Wheatgrass supplementation has been shown to lower the level of oxidative stress markers and improve hematological parameters in individuals with chronic diseases (Ben-Arye et al., 2015). Furthermore, comparative studies report the existence of catalase, peroxidase, and superoxide dismutase in wheatgrass, which exert marked antioxidant properties (Singh et al., 2012; Sharma & Sunkad, 2015).

Polyherbal Synergy

The synergism principle of phytotherapy holds that the interaction of various constituents in a plant will increase the therapeutic effects of the plant. Wagner (2011) reported that phytochemical synergy may lie within multi-target activity, increased bioavailability, or perpetuated antioxidant action. Many polyherbal formulations showed better antioxidant and immunomodulatory effects than single plants (Patwardhan et al., 2015). Further, recent investigations have shown that synergistic effects of bioactive plant extracts exhibit an improved free radical scavenging and enzyme inducing activities as compared to their active compounds alone (Khushboo et al., 2022).

Rationale for Neem & Wheat Combination

Phenolics and flavonoids with potent free radical scavenging activity are present in neem (Alzohairy, 2016; Subapriya et al., 2005) whereas chlorophyll, phenolic acids and endogenous anti-oxidant enzymes are available in wheat grass (Kulkarni et al., 2006; Singh et al., 2012). Accordingly, a combination of such extracts should give synergistic effects, Neem having strong radical quenching and Wheatgrass fortifying the enzymatic antioxidant defense. But, systematic reviews of Neem, Wheat synergy were not published in the references, which is an indication of the need to fill a research gap.

METHODOLOGY

Plants

The fresh leaf sample of Azadirachta indica (Neem) was collected from the botanical garden of the Islamia University of Bahawalpur, Pakistan, fresh young shoots of Triticum aestivum (Wheatgrass) were grown in controlled conditions and cut after 10 to 12 days of seed germination. Both the plants were identified by a taxonomist of the Department of Botany and voucher specimens were

lodged in the herbarium of the department for future reference. The harvested materials were washed properly and air-dried in shade at room temperature followed by grinding to a fine powder using electrical grinder. The powdered samples were stored in refrigerator at 4 °C in sealed container.

Figure 1

Plant Materials (a) Azadirachta indica (Neem)

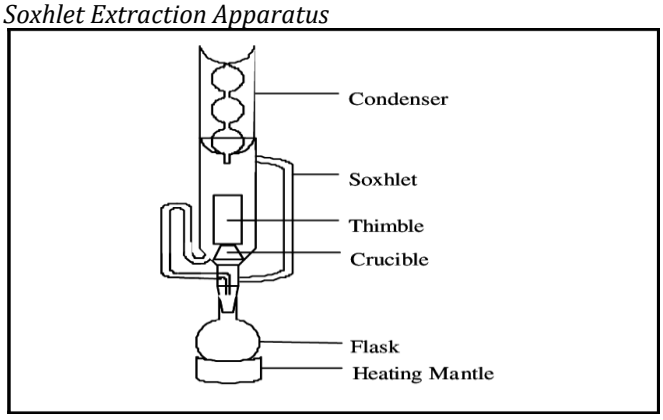
(b) Triticum aestivum (Wheatgrass)

Preparation of Extracts

Powdered samples (50 g each) of Neem (Azadirachta indica) leaves and Wheatgrass were subjected to successive extraction by Soxhlet apparatus for 8–10 hours with methanol and ethanol, until the solvent in the siphon tube turns colorless. The extracts were filtered by using Whatman No. 1 filter paper, and later on evaporated under reduced pressure at 40 °C on a rotary evaporator. The crude extracts were pressed/flasked, weighed and kept in air tight bottles at 40C for further analysis. For synergism studies, extracts of Neem and Moringa were mixed in 1:1 weight/weight ratio before phytochemical and enzyme assays.

Figure 2

Soxhlet Extraction Apparatus



Qualitative Phytochemical Screening

A preliminary phytochemical analysis was conducted in accordance with standard procedures to determine the presence of alkaloids, flavonoids, tannins, saponins, terpenoids, phenolics, and glycosides (Harborne, 1998; Trease & Evans, 2002).

Quantitative Estimation of Phytochemicals

Total Phenolic Content (TPC): Measured in mg GAE/g extract with the Folin–Ciocalteu method.

Total Protein: It determined by the Lowry method.

Antioxidant Enzyme Assays

Catalase (CAT) Activity: It was determined by the decomposition of H_2O_2 at 240 nm and was expressed as U/mg protein (Aebi, 1984).

Peroxidase (POD): As per guaiacol oxidation at 470 nm, in U/mg protein.

Superoxide Dismutase (SOD) Activity: It measured by nitroblue tetrazolium (NBT) reduction and expressed as U/mg protein.

Statistical Analysis

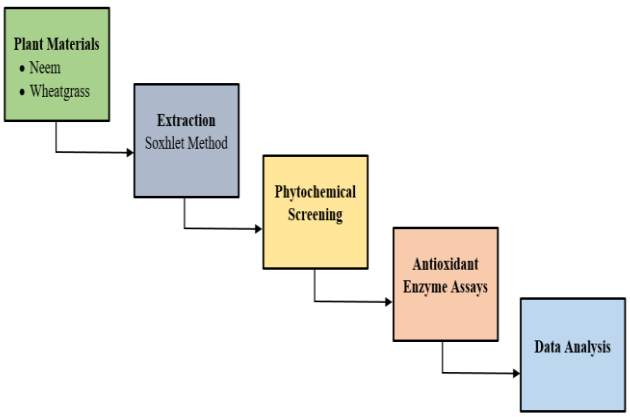
Each experiment was repeated three times, and data were recorded as mean ± SD. The data were analyzed with one-way ANOVA and, if necessary, Tukey's post-hoc test for significance ($p < 0.05$).

Workflow of the Experiment

Flow chart of the experimental methods used in the study, from the collection of plant materials of Azadirachta indica (Neem) and Triticum aestivum (Wheatgrass), followed by Soxhlet extraction, phytochemical analysis (qualitative and quantitative), anti-oxidant enzyme activity (CAT, POD, SOD), and final statistical analysis by mean of ANOVA and Tukey's post hoc test.

Figure 3

Workflow



RESULTS

Qualitative Phytochemical Screening

Preliminary phytochemical screening revealed the existence of alkaloids, flavonoids, tannins, phenols and saponins in Neem and Wheatgrass extracts. Neem contained relatively higher levels of terpenoids and glycosides, while Wheatgrass was rich in chlorophyll related compounds. Combination of Neem & Wheat extract revealed nearly all phytochemical groups which implied synergistic interactions. These findings are in accordance

with previous findings which indicated that Neem is known to have abundant limonoids and flavonoids (Subapriya & Nagini, 2005), and that Wheatgrass contains chlorophyll, phenolics, and vitamins (Kulkarni et al., 2006).

Table 1

Qualitative Phytochemical Screening of Neem, Wheatgrass, and Combined Extracts

Phytochemical	Neem Extract	Wheatgrass Extract	Combined Extract
Alkaloids	+++	++	+++
Flavonoids	++	++	+++
Tannins	++	+	++
Phenolics	++	++	+++
Saponins	+	+	++
Terpenoids	+++	+	+++
Glycosides	++	+	++

(+ weak; ++ moderate; +++ strong presence)

Quantitative Estimation of Phenolics and Proteins

The Total Phenolic Content: The TPC of combined extract was significantly higher than that Neem (54.28 mg GAE/g) Wheatgrass (61.34 mg GAE/g) ($p < 0.05$).

Protein Self-Assembly: The protein content of the synergistic extract (98.72 mg/g) was higher than that of Neem (69.40 mg/g) and Wheatgrass (72.19 mg/g).

This doubling shows an additive or synergistic effect when the two plants are mixed together.

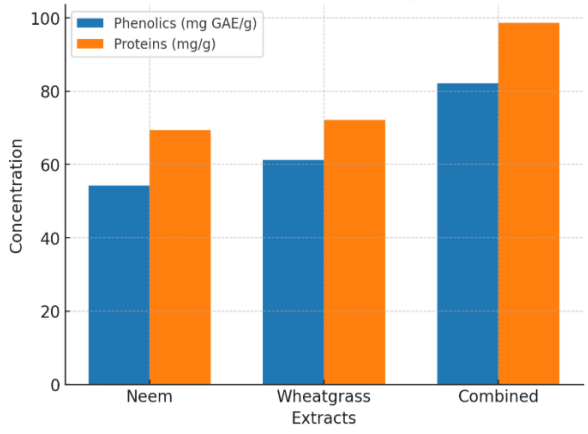
Table 2

Quantitative Estimation of Phenolics and Proteins in Neem, Wheatgrass, and Combined Extracts

Sample	Total Phenolics (mg GAE/g)	Protein Content (mg/g)
Neem Extract	54.28 ± 2.1	69.40 ± 1.8
Wheatgrass Extract	61.34 ± 1.9	72.19 ± 2.0
Combined Extract	82.14 ± 2.4	98.72 ± 2.2

Figure 4

Phenolic and Protein Content



Antioxidant Enzyme Activities

Peroxidase (POD), Catalase (CAT) and Superoxide Dismutase (SOD) activities from the combined extracts were significantly higher than their corresponding single plant extracts. CAT Activity in combined extract was greater, 28.42 U/mg, compared to both Neem, 19.36 U/mg, and Wheatgrass, 21.48 U/mg. Anti-oxidative enzymes such as POD and SOD also increased, indicating a synergistic improvement in enzymatic anti-oxidative defense.

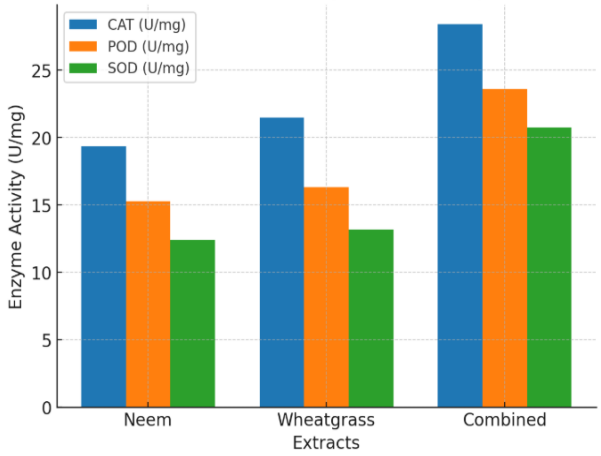
Table 3

Antioxidant Enzyme Activities in Neem, Wheatgrass, and Combined Extracts.

Sample	CAT (U/mg)	POD (U/mg)	SOD (U/mg)
Neem Extract	19.36 ± 0.9	15.27 ± 0.7	12.42 ± 0.5
Wheatgrass Extract	21.48 ± 0.8	16.34 ± 0.6	13.18 ± 0.4
Combined Extract	28.42 ± 1.0	23.61 ± 0.9	20.74 ± 0.7

Figure 5

Antioxidant Enzyme Activities (CAT, POD, SOD)



DISCUSSION

Significant increase in phenolics, proteins, and antioxidant enzyme activities were found in the combination extracts of Neem and Wheatgrass, as compared to their individual extracts. This is consistent with the conclusion that the combination of phytochemically diverse vegetables contributes to antioxidant potential by complementary mechanisms. Neem-based flavonoids and limonoids (Subapriya & Nagini, 2005; Alzohairy, 2016) and Wheatgrass offer radical scavenging (Singh et al., 2012) whose summation resuscitates CAT, POD, and SOD activities. This synergy is in concordance with the action of polyherbal principles enunciated by Wagner (2011) as a multi-targeted phytochemical association for potentiation of biological activity. These findings indicate that different from single-plant preparations, Neem & Wheat formulations may offer more efficient protection against oxidative stress.

CONCLUSION

In the present investigation, a strong synergistic interaction between A. indica (Neem) and T. aestivum (Wheatgrass) extracts are observed with higher phytochemical content and antioxidant enzyme activities as opposed to their respective individual extracts. The composite extract also had greater amounts of total phenolics and proteins that were associated with increased catalase, peroxidase and superoxide dismutase activities. These results favour the notion of phytochemical synergy, where various bioactive substances interact additively or synergistically yielding a better antioxidant potential.

The findings corroborate Neem & Wheat formulations may represent potential candidates for nutraceutical and therapeutic utility for the management of oxidative stress

associated disorders. Nevertheless, additional in vivo and clinical experiments are needed to verify these synergistic actions, and to investigate their potential applications in functional food or herbal medicine.

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