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PHYTOCHEMICAL SYNERGY: ANTI-INFLAMMATORY & ANTIOXIDANT EFFECTS OF *PANAX GINSENG, GINKO BILOBA* & *ASTRAGALUS MEMBRANACEUS*

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ABSTRACT

Aim: The aim of the present study is to find out the Anti-inflammatory & Antioxidant effects of *Panax ginseng*, *Ginko biloba* & *Astragalus membranaceus.* **Material and Methods:** All the plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of powder of *Panax ginseng*, *Ginkgo biloba* and *Astragalus membranaceus* were extracted using 95 % ethanol by soxhlet apparatus for 72 h. Qualitative chemical tests of ethanolic extracts were subjected to various chemical tests to detect various phytoconstituents. Acute oral toxicity studies were conducted following the revised draft guideline 423 of the Organization for Economic Co-operation and Development (OECD). Inflammation was induced by 0.1 mL of 1% suspension of carrageenan into hind paw of rat by sub planter route. Treatments of all fractions were given 1 h prior to administration of carrageenan. Paw volume was measured with digital plethysmograph at 0, 1, 2, 3, 4 and 5th h after injection. The DPPH method refers to the method used by Irawan.13 An amount of 5 mg of extract was dissolved with ethanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1,000 mg/L. **Results:** The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds. The ethanolic extracts significantly (p<0.01, p<0.001) inhibited carrageenan induced rat paw edema as compared to control group. Maximum inhibition of rat paw edema was observed with ethanolic extracts of all plants at the end of 5th h when compared to control group. Indomethacin (10 mg/kg) inhibited the paw edema. When free radicals react with antioxidants, their free radical properties are lost because the chains are broken and the color changes from purple to light yellow. The results of the antioxidant activity test with DPPH were expressed as % inhibition. **Conclusion:** In summary, ethanolic extract of *Astragalus membranaceus* demonstrated significant therapeutic effects by consistently reducing rat paw edema.

KEYWORDS: Phytochemical Synergy, Anti-inflammatory & Antioxidant Effects, *Panax ginseng*, *Ginko biloba* & *Astragalus membranaceus.*

INTRODUCTION

Phytochemicals found in medicinal plants serve as the foundation for numerous therapeutic agents used to treat a wide range of health conditions, and they continue to be vital resources for novel drug discovery and development (Bachheti et al., 2020; Asmamaw & Achamyeleh, 2018). In many developing nations, medicinal plant products remain the most accessible and

affordable option for primary healthcare (Gumisiriza et al., 2019). The primary advantage of plant phytochemistry lies in its application of plant-derived substances as treatments for various ailments. Due to their low incidence of side effects, these plant-based remedies are widely used for both human and livestock diseases and maintain strong community acceptance (Kerdar et al., 2019). Consequently, the use of natural plant products as alternative medicines for managing diverse health issues is on the rise. According to the World Health Organization, over 20,000 species of medicinal plants have been cataloged to date (Vaou et al., 2021). Additionally, some plant-derived products are utilized as nutraceuticals (Mbendana et al., 2019).

The study of medicinal plants has uncovered numerous secondary metabolites with pharmaceutical applications, with approximately 50% of modern medications derived from these natural substances (Sharma et al., 2017). Phytochemicals such as phenolic compounds, saponins, proanthocyanidins, nitrogenous compounds, alkaloids, and terpenoids have demonstrated notable pharmacological potential (Bunte et al., 2019). Currently, plant-based phytochemicals and their associated nanoparticles are key areas of research due to their diverse and extensive biological applications (Gonfa et al., 2021). Moreover, advancements in green synthesis of metal and metal oxide nanoparticles (M/MONPs), which possess at least one dimension below 100 nm, have shown promise for their toxicity against harmful pathogens. Phytochemicals play essential roles in the green synthesis of M/MONPs, acting as reducing, stabilizing, and capping agents (Zhang et al., 2021). The combined effects of metal ions and phytochemical capping agents contribute significantly to their strong disease-fighting capabilities. Over the past decade, numerous in vitro studies have confirmed the pharmacological efficacy of plant-derived phytochemicals and their nanoparticles. Inflammation is a natural biological response that helps cells defend themselves against infections, harmful agents, and immune dysfunction. It can be classified as acute (initial protective response) or chronic (prolonged and potentially damaging) (Sumathi & Anuradha, 2016). Typical signs of inflammation include redness, swelling, heat, pain, and impaired function (Owolabi et al., 2018). In response to stimuli such as microbial infections, mechanical injuries, or burns, the body's cells activate defense mechanisms (Anyasor et al., 2019). Both acute and chronic inflammatory processes are vital components of the innate immune response, essential for maintaining human health (Oguntibeju, 2018).

The literature review highlights Panax ginseng, Ginkgo biloba, and Astragalus membranaceus as commonly used medicinal plants in managing acute and chronic inflammation. These plants have also been noted for their immunomodulatory properties. However, due to limited scientific evidence supporting their anti-inflammatory and antioxidant activities in such conditions, efforts are being made to validate these properties. The goal is to identify effective and potent bioactive phytoconstituents with fewer side effects than current synthetic drugs.

MATERIALS AND METHODS

Plant Materials

The *Panax ginseng*, *Ginkgo biloba* and *Astragalus membranaceus* were collected in the month of March from campus of College of Pharmacy.

Authentification of Plant Materials

All the plant materials were taxonomically identified by Senior Scientist, College of Horticulture. The herbarium sheets were submitted in Department of Pharmacognosy under voucher specimen.

Preparation of Total Crude Extract

All the plant materials were dried under shade and subjected to coarse powder for extraction process. Accurately weighed quantity of powder of *Panax ginseng*, *Ginkgo biloba* and *Astragalus membranaceus* were extracted using 95 % ethanol by soxhlet apparatus for 72 h. The ethanolic extracts were dried completely under reduced pressure. After drying, the respective extracts were weighed and percentage yield was determined (Mukherjee, 2002).

Preliminary Phytochemical Tests

Qualitative chemical tests of ethanolic extracts were subjected to various chemical tests to detect various phytoconstituents (Kokate, 2003; Khandelwal, 2006).

Selection of animals

Wistar albino rats of either sex, aged between 2 and 3 months and weighing between 150–200 g, were utilized in the study. The animals were obtained from the central animal facility of the College of Pharmacy, India. They were housed under standard laboratory conditions, maintained at a temperature of 25±1°C with a 12-hour light/dark cycle. Rats had unrestricted access to a commercial pellet diet (Lipton India Ltd, Mumbai, India) and water. Cage bedding was replaced daily to maintain hygiene. All experimental procedures adhered to the guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), and the study protocol was approved by the Institutional Animal Ethical Committee of the College of Pharmacy.

Acute toxicity studies

Acute oral toxicity studies were conducted following the revised draft guideline 423 of the Organization for Economic Co-operation and Development (OECD). Based on the lethal dose, one-tenth and one-fifth of the determined value were selected as the effective (therapeutic) doses. Accordingly, 200 mg/kg and 400 mg/kg were chosen as the cut-off doses to assess the dose-dependent effects in the evaluation of anti-arthritic activity (OECD guidelines, 2001).

Anti-inflammatory activity of ethanolic extracts in carrageenan induced rat paw edema

Animals were divided into 9 groups and each group contains 6 animals. The animal groups and treatment schedule are as follows.

Group-I: Normal control treated with normal saline (5 ml/kg p.o.)

Group-II: Disease control treated with 0.1 mL of 1% carrageenan in 0.9% saline

Group-III: Standard control treated with Indomethacin (10 mg/kg p.o.) + Carrageenan

Group-IV: Ethanolic extract of *Panax ginseng* (200 mg/kg p.o.) + Carrageenan

Group-V: Ethanolic extract of *Panax ginseng* (400 mg/kg p.o.) + Carrageenan

Group-VI: Ethanolic extract of *Ginkgo biloba* (200 mg/kg p.o.) + Carrageenan

Group-VII: Ethanolic extract of *Ginkgo biloba* (400 mg/kg p.o.) + Carrageenan

Group-VIII: Ethanolic extract of *Astragalus membranaceus* (200 mg/kg p.o.) + Carrageenan

Group-IX: Ethanolic extract of *Astragalus membranaceus* (400 mg/kg p.o.) + Carrageenan

Inflammation was induced by 0.1 mL of 1% suspension of carrageenan into hind paw of rat by sub planter route. Treatments of all fractions were given 1 h prior to administration of carrageenan. Paw volume was measured with digital plethysmograph at 0, 1, 2, 3, 4 and 5th h after injection. The inhibitory activity was calculated using the following formula (Mali *et al*., 2013).

Percentage inhibition= Vc-Vt/Vc ×100

Where,

Vc- Paw volume of control rat

Vt- Paw volume of treated rat

Antioxidant activity test using 1,1-diphenyl2-pikrilhidrazil (DPPH) method

The DPPH method refers to the method used by Irawan.13 An amount of 5 mg of extract was dissolved with ethanol pa in a 5 mL measuring flask, resulting in a sample solution with a concentration of 1,000 mg/L. DPPH 39 mg/L solution was added to five 5 mL measuring flasks, then each was put into five 5 mL measuring flasks, then each was added 2 mL of DPPH 39 mg/L solution, then measured with methanol pa, and homogenized (sample concentrations of 40, 80, 160,320 and 640 mg/L). The solution was incubated for 30 minutes at room temperature (25o C), then the absorbance of the solution was measured using a visible light spectrophotometer at a wavelength of 515 nm. The work is carried out in five repetitions. The same work was carried out to make a comparison solution of BHT with concentrations of 2, 4, and 8 mg/L. Antioxidant activity is expressed as percent inhibition (% inhibition) with the following equation.

$$\%\ Inhibition = \frac{(A_{blank} - A_{sample})}{A_{blank}} x100\%$$

Details

A_{blank}=Absorbance without sample

A_{sample}=Absorbance of the sample

STATISTICAL ANALYSIS

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p< 0.05$ was chosen as the level of significance.

RESULTS

Extractive Value Determination

Dried parts of *Panax ginseng*, *Ginkgo biloba* and *Astragalus membranaceus* were extracted using ethanol. The percentage yields of all dried extracts were determined by using the following formula.

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

Table No 1: Different extracts with their appearance and % yield (in gm).

S. No.	Extracts	Colour of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Ethanolic extracts of *Panax ginseng*	Dark Green	Sticky	26 %
2	Ethanolic extracts of *Ginkgo biloba*	Dark Green	Sticky	28 %
3	Ethanolic extracts of *Astragalus membranaceus*	Dark Orange	Sticky	21 %

Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds.

Table No 2: Qualitative chemical analysis of extracts by chemical tests.

S. No	Phytoconstituents	Chemical Tests	*Panax ginseng*	*Ginkgo biloba*	*Astragalus membranaceus*
1	Alkaloids	Wagner's test	+	+	-
		Dragendorff's test	+	+	-
		Mayer's test	+	+	-
		Hager's test	-	-	-
2	Amino Acid	Millon's test	+	+	-
		Ninhydrine test	-	-	-
3	Flavonoids	Shinoda test	+	+	+
		Alkaline reagent test	+	+	+
		Zinc hydrochloride test	_	+	+
4	Phenolics (Tannins)	Gelatin test	+	+	+
		Phenazone test	-	-	-
		Ferric chloride test	+	+	+
5	Protein	Biuret test	+	+	-
		Hydrolysis test	+	+	+
		Test with trichloroacetic acid	-	-	-
6	Triterpenoids & Steroids	Libermann-Burchard test	+	+	+
		Salkowski test	+	+	+
7	Carbohydrates	Benedict's test	+	+	+
		Fehling's test	+	+	+
		Molish's test	-	-	-
8	Anthraquinone glycosides	Borntrager's test	+	+	+
		Modified Borntrager's test	+	+	+
9	Coumarin glycosides	_____	-	-	-
10	Saponin glycosides	_____	+	+	+
11	Cardiac glycosides	Baljet's test	+	+	+
		Legal's test	+	+	+
		Keller-killiani test	+	+	+

Where, (-) Negative, (+) Positive

Acute Toxicity Studies of Plant Extracts

No toxic effects were observed at a higher dose of 2000 mg/kg body weight of Wistar rats. Hence, 1/ 10th dose was selected as effective dose or therapeutic dose. The cut off value of 200 and 1/5 dose double of 400 mg/kg were selected for anti-inflammatory activity.

Table No 3: Acute toxicity studies of plant extracts.

S. No.	Treatment	Dose (mg/kg)	Number of animals	Mortality			Toxicity Profile
				After 24 hrs	After 7 days	After 14 days	
1	*Panax ginseng*	2000	5	0	0	0	Safe
2	*Ginkgo biloba*	2000	5	0	0	0	Safe
3	*Astragalus membranaceus*	2000	5	0	0	0	Safe

Anti-inflammatory activity of Ethanolic Extracts

The ethanolic extracts significantly ($p<0.01$, $p<0.001$) inhibited carrageenan induced rat paw edema as compared to control group. Maximum inhibition of rat paw edema was observed with ethanolic extracts of all plants at the end of 5^{th} h when compared to control group. Indomethacin (10 mg/kg) inhibited the paw edema.

Table No 4: Effects of ethanolic extracts of different plants in rat paw edema model.

S. No.	Groups & Treatments	"0"Min	1 h	2 h	3 h	4 h	5 h
1	Normal Control	0.23±0.22	0.22±0.18	0.22±0.20	0.22±0.19	0.23±0.10	0.23±0.22
2	Disease Control, 1% Tween 80, p.o.	0.23±0.22	0.36±0.01	0.42±0.03	0.58±0.02	0.67±0.03	0.62 ± 0.04
3	Ethanolic extract of *Panax ginseng*	0.24±0.03	0.33±0.01	0.40±0.04	0.50±0.04*	0.62±0.02*	0.62±0.04
4	Ethanolic extract of *Panax ginseng*	0.23±0.05	0.32±0.03	0.39±0.03	0.49±0.07*	0.60±0.07*	0.59±0.08
5	Ethanolic extract of *Ginkgo biloba*	0.25±0.04	0.32±0.03	0.33±0.06**	0.38±0.02**	0.41±0.08**	0.44±0.01**
6	Ethanolic extract of *Ginkgo biloba*	0.23±0.07	0.28±0.04	0.0±0.09**	0.35±0.04**	0.38±0.02**	0.39±0.07**
7	Ethanolic extract of *Astragalus membranaceus*	0.22±0.02	0.30±0.02*	0.32±0.04**	0.36±0.03***	0.36±0.04***	0.38±0.06***
8	Ethanolic extract of *Astragalus membranaceus*	0.22±0.08	0.27±0.04*	0.28±0.03**	0.31±0.07***	0.30±0.08***	0.28±0.03***
9	Indomethacin10 mg/kg, p.o.	0.25±0.03	0.27±0.03**	0.28±0.06***	0.30±0.07***	0.31±0.07***	0.32±0.04***

Values are expressed as mean±SEM, n=6 in each group; *p<0.05, compared to disease control ** p<0.01, compared to disease control. ***p<0.001, compared to disease control

Anti-oxidant activity of the DPPH Method

Antioxidants are substances that can slow down or prevent the oxidation process. This substance is significantly able to slow down or inhibit the oxidation of substances that are easily oxidized even in low concentrations. DPPH is a free radical which is stable at room temperature and in methanol and produces a purple solution. When free radicals react with antioxidants, their free radical properties are lost because the chains are broken and the color changes from purple to light yellow. The results of the antioxidant activity test with DPPH were expressed as % inhibition.

Table No 5: Effects of ethanolic extracts of different plants in DPPH Method.

Sample	Concentration (mg/ml)	% Inhibition
	2	34.77±0.09
BHT	4	51.65±0.05
	8	71.22±0.07
	40	3.30±0.04
	80	6.04±0.05
Panax ginseng	160	13.22±0.04
	320	26.24±0.02
	640	50.07±0.08
	40	6.93±0.02
	80	8.78±0.08
Ginkgo biloba	160	15.91±0.05
	320	29.04±0.04
	640	50.76±0.01
	40	10.18±0.07
	80	16.79±0.06
Astragalus membranaceus	160	23.76±0.02
	320	30.78±0.05
	640	54.14±0.03
	640	50.21±0.05

DISCUSSION

Phytochemical screening revealed that all extracts contained bioactive compounds such as steroids, terpenoids, alkaloids, flavonoids, glycosides, and fatty acids. Acute oral toxicity assessments were carried out for ethanolic extracts of all plants and they did not induce any signs of toxicity, such as hypersensitivity, diarrhea, itching, behavioral alterations, or mortality, at a dose of 2000 mg/kg body weight. Consequently, 200 mg/kg and 400 mg/kg (1/10th and 1/5th of the lethal dose, respectively) were selected as therapeutic doses for further evaluation of anti-inflammatory activities. For assessing acute inflammation, all extracts were tested using the carrageenan-induced rat paw edema model. The ethanolic extract of *Astragalus membranaceus* displayed the most pronounced activity. The

carrageenan-induced rat paw edema model is a widely used in vivo method to evaluate the anti-edematous properties of natural compounds, known to be sensitive to cyclooxygenase (COX) inhibitors and thus suitable for testing non-steroidal anti-inflammatory drugs (NSAIDs). Carrageenan, a sulfated polysaccharide derived from the red seaweed *Chondrus crispus*, induces inflammation via a multi-phase process involving various inflammatory mediators. The initial (0–2 hours) phase is mediated by the release of histamine, serotonin, and bradykinin, followed by a prostaglandin-driven third phase, during which maximum edema occurs, typically by the third hour, and begins to subside thereafter. In our study, all tested fractions and the standard drug indomethacin significantly reduced inflammation in this model. Previous research has shown that maximum edema occurs at the third hour, driven by prostaglandins and slow-reacting inflammatory compounds (Kirkova et al., 1992; Spector & Willoughby, 1963). The anti-inflammatory effects observed in our experiment began from the second hour and peaked around the fifth hour, suggesting inhibition of early-phase mediators like histamine and serotonin, as well as late-phase agents such as bradykinin and prostaglandins.

Based on the results from the antioxidant activity test methods, the DPPH method showed a distinct response. It is assumed that the extract contains antioxidant compounds capable of reducing Fe(III)-TPTZ under thermodynamically favorable conditions and with a relatively rapid reaction rate. Furthermore, the oxidized antioxidants and their secondary reaction products must exhibit maximum absorbance at the same wavelength as Fe(III)-TPTZ. The antioxidant potential of plant-derived substances is commonly linked to the presence of phenolic and flavonoid compounds. Phenolic compounds are known for their antioxidant activity due to their redox properties. They function as reducing agents, hydrogen donors, singlet oxygen quenchers, and potential metal ion chelators. Similarly, flavonoids act as antioxidants by neutralizing free radicals through hydrogen atom donation.

CONCLUSION

In summary, ethanolic extract of *Astragalus membranaceus* demonstrated significant therapeutic effects by consistently reducing rat paw edema. Their mode of action is likely linked to the inhibition of prostaglandin synthesis during acute inflammation and suppression of lysosomal enzyme activity during chronic inflammatory responses, supporting the traditional claims made in Siddha and Ayurveda medicine. They may serve as promising lead extract in the development of new anti-inflammatory drugs. However, further in-depth studies are necessary to clarify their exact mechanisms of action, particularly at the molecular and genetic levels. The present results are important for guiding the development of alternative, cost-effective, and potentially safer treatment strategies.

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