Preparation of *Solanum Virginianum (L.)* And *Acacia Nilotica (L.)* Leaves Methanolic Extracts and Assessment of Their Anti-Inflammatory and Anti-Oxidant Potentials

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Abstract:
Inflammation is a reaction of the immune system that occurs in the context of some type of physical damage or infection. Chronic inflammatory diseases develop when normally self-limiting inflammatory processes become chronic. The increased flow of blood and cell metabolism, extravasation of fluid, release of soluble mediators, vasodilatation and influx of cells are all parts of the immune nonspecific response which occurs in response to any form of physiological injury and is the primary marker of inflammation. This study aimed to investigate the anti-inflammatory and anti-oxidant potentials of *Solanum virginianum* (L.) and *Acacia nilotica* (L.) leaves Methanolic extracts, using in vitro assays (Human Red Blood Cell (HRBC) membrane stabilization assay, Heat-Induced Hemolysis (HIH) Assay and DPPH Scavenging assay).The results showed that the stabilization of HRBC by a 100µg/ml dose of *S. virginianum* were 75±1.33% While the stabilization of HRBC by *A. nilotica* was 78±2.05%. The HRBC also showed high heat tolerance of 72±1.8% protection with *S. virginianum* and that of *A. nilotica* was 76±1.93% at a dose of 100µg/mL. In the antioxidant activity of *S. virginianum* and *A. nilotica* scavenging rates were 71.01±0.43 and 75.31±0.55% respectively. In conclusion, *S. virginianum* and *A. nilotica* exhibit high anti-inflammatory and antioxidant activities. The results of this study demonstrate that the investigated plants have strong anti-inflammatory and anti-oxidant potentials.

Keywords: Anti-inflammatory; Anti-oxidant; *Solanum virginianum; Acacia nilotica*; natural products

Introduction

Inflammation is a reaction of the immune system that occurs in the context of some type of physical damage or infection. Chronic inflammatory diseases develop when normally self-limiting inflammatory processes become prolonged. The increased flow of blood and cell metabolism, extravasation of fluid, release of soluble mediators, vasodilatation and influx of cells are all parts of the immune nonspecific response which occurs in response to any form of physiological injury and is the primary marker of inflammation [1]. However, unchecked acute inflammation can last for a long time and contribute to several chronic inflammatory diseases. Inflammation plays a significant role in the pathophysiology of various diseases, including diabetes, neurodegenerative cardiovascular diseases and cancer [2, 3]. Chronic and acute inflammatory illnesses can lead to uncontrolled inflammatory responses. Therefore, anti-inflammatory medications must be taken to manage the immunological response [4, 5]. However, due to the serious side effects of NSAIDs, including damage to the gastrointestinal system, kidneys and heart there is a significant reliance on the use of natural substances [6].The conventional signs of inflammation include swelling, redness, pain and fever. These symptoms are due to various inflammatory chemical agents and mediators, for example histamine, prostaglandins (PGs), serotonin, leukotrienes, bradykinin and nitric oxide which are produced either locally or infiltrate the tissue of inflammation [7].

The word ‘antioxidant’ describes all those vitamins, minerals, phytochemicals, and numerous compounds that give defense against impairment caused by Reactive Oxygen species [8]. Antioxidants perform their activity by interfering with the process of oxidation, they scavenge the free radicals, chelate the metallic ions that are free by giving them their own electrons thus acting as donors of electrons [9]. There is a strong link between taking diet rich in antioxidants and the incidence of diseases of human caused by antioxidants [10].

Anti-inflammatory compounds from natural sources have been extensively studied and have shown hopeful results against inflammatory disorders due to their minor side effects [11]. *Solanum virginianum L*. is a member of the Solanaceae family widely distributed throughout Asia [12, 13]. Different parts of the plant contain phenolics, alkaloids, sterols, flavonoids, saponins, fatty acids, glycosides, tannins and amino acids, among other phytochemicals [14]. The herb is widely used in

different types of medicine; diosgenin, Diosgenin, β-sitosterol, carpesterol and some others like *Solasonine, Solamargine*, B2-Solamargine, Solasodine, caffeine, and Oleanolic Acid compounds have been reported from this plant [15, 16].

The current study aims to investigate the anti-inflammatory and anti-oxidant potentials of Methanolic extracts of *S. virginianum* and *A. nilotica* using in vitro assays. The results of this study will provide insights into the anti-inflammatory and anti-oxidant mechanisms of these extracts and their potential as therapeutic agents for the treatment of inflammation.

Materials and Methods

Plants collection

Both S. *virginianum* and *A. nilotica* plants were collected from the Khyber Pakhtunkhwa region of Pakistan and identified in the herbarium of the Botany Department with specimen no. Bot/2021/0240 and Bot/2021/0241, respectively, at Abdul Wali Khan University Mardan (AWKUM), Khyber Pakhtunkhwa, Pakistan.

Preparation of Plants Extracts

The leaves of both plants were separated and cleaned individually. After that, they were dried and ground into a fine powder. Both types of powders were extracted separately using two different methods. The first method involved macerating the powders in Methanol (1:2 W/V) for a period of 5 days. The resulting mixture was then filtered and concentrated using a rotary evaporator. This process was repeated twice on the residue and the final extract was labeled as a Methanolic extract.

In Vitro Anti-Inflammatory Activity

Human Red Blood Cell (HRBC) membrane stabilization assay

The in vitro anti-inflammatory potentials of both plants were studied by a human red blood cell (HRBC) membrane stabilization assay following the method of HRBCs [17]. HRBCs Suspension was prepared by freshly collecting 5 mL of human blood from a healthy adult donor who had not taken any anti-inflammatory medication in the preceding ten days, transferring it to 15.0 mL tubes, and centrifuging at 3000 RPM for 5 minutes. The HRBC pellet was washed three times with an isosaline solution. The percentage of hemolysis protection was calculated by the following formula:

$$\text{Percent of protection} = \text{Control} - \text{Test sample}/\ \text{control} \times 100$$

Heat-Induced Hemolysis (HIH) Assay

This assay was performed by following the method of HRBCs with minor modifications [18]. The Percent of Hemolysis level was calculated by the following equation.

$$\text{Percent of hemolysis inhibition} = (1 - B1/B2) \times 100$$

Whereas: B1 = control and B2 = absorbance of sample

Antioxidant Assays performed In vitro:

DPPH Scavenging assay:

The capability of both plant extracts for free radical scavenging was tested using DPPH, according to [19]. The DPPH solution was utilized in this experiment. Both plant extracts were utilized to determine the antioxidant activity. 1 mg of DPPH powder was mixed with 25 ml of methanol to make a 0.1 mM DPPH solution. For each experiment, a new DPPH solution is prepared. 0.1 mM DPPH in a methanol solution was utilized in the DPPH scavenging experiment. In this experiment, 3 milliliters of reaction sample were combined with 1 milliliter of DPPH and various concentrations of both plants. In reaction mixes that have been stored in the dark. As a control, ascorbic acid (vitamin C) at concentrations of 20, 40, 60, 80, and 100 ug/ml was added to the reaction mixture. The reaction sample was incubated for 30 minutes at 37ºC before the spectrometer absorbance was set at 517 nm. As the free radical scavenging activity increased, the absorbance value of the reaction mixture decreased.

The percent scavenging of the DPPH radical was determined using the following formula:

$$\text{Scavenging percentage} = \frac{\text{absorbance of control group - absorbance of sample}}{\text{Absorbance of Control group}} \times 100$$

Results

Human Red Blood Cell hemolysis Stabilization (HRBCs)

Stabilizing human red blood cells HRBCs assay Results

The in vitro anti-inflammatory potentials of the of *S. virginianum* and *A. nilotica* leaves showed good results, with 75±1.33% and 78±2.05% stabilization of hemolysis at 100 µg/mL respectively (Figures 1). Diclofenac indicated relatively maximum anti-inflammatory activity (84%±0.54%) at 100 µg/ml. In this study, *S. virginianum* and *A. nilotica* showed better in vitro anti-inflammatory activity in a dose-dependent manner in inhibition of hemolysis (Figure 1).

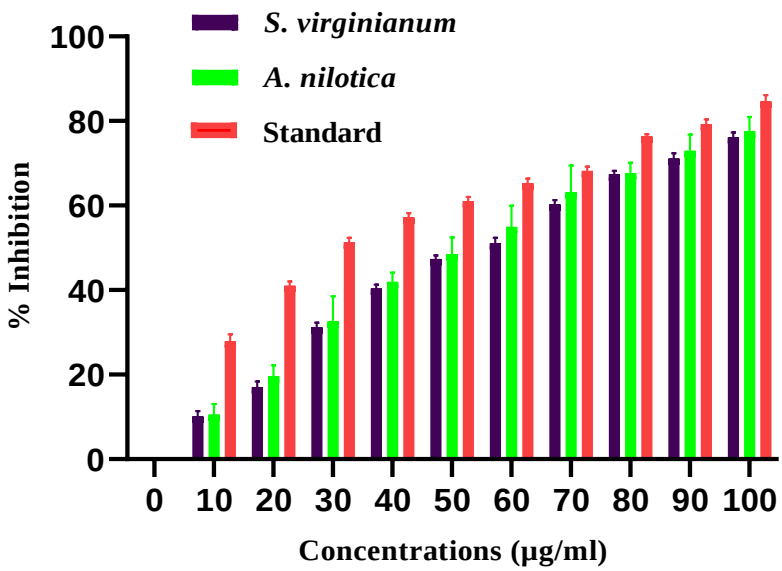

Figure 1. *S. virginianum, A. nilotica* against Human Red Blood Cell Stabilization (HRBCs) in terms of % inhibition.

Stabilization of Heat-induced hemolysis of HRBCs membrane

Effect of *S. virginianum* and *A. nilotica* on the heat-induced hemolysis of HRBCs

The prevention of heat-induced hemolysis of HRBC by various doses of the *S. virginianum* and *A. nilotica* was maximum 72±1.08% and 76±1.93% respectively, at 100 µg/ml compared to the standard drug that prevented 84±1.37% (Figure 2). The findings suggest that *S. virginianum* and *A. nilotica* can effectively prevent hemolysis.

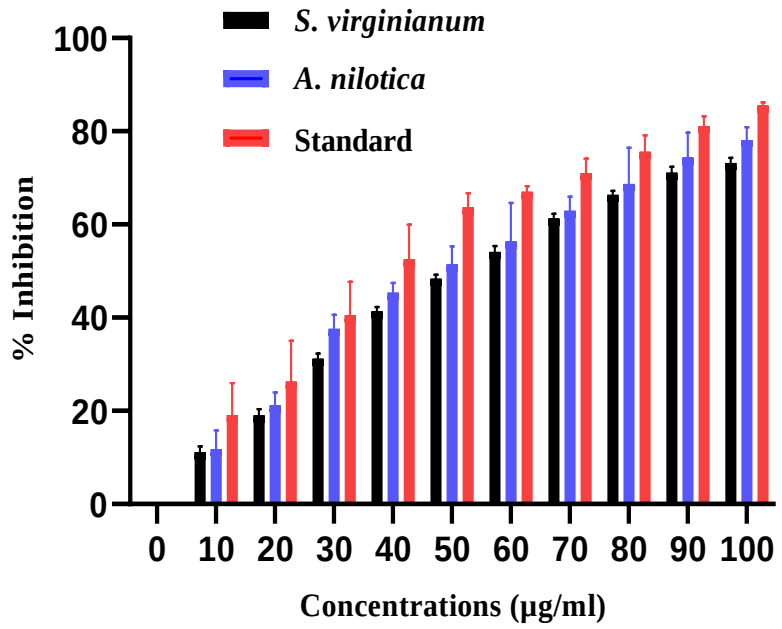

Figure 2. Shows Effect of various doses of *S. virginianum, A. nilotica* on Heat-induced Hemolysis of HRBCs membrane

Antioxidant Activity Results

DPPH scavenging assay Results

The study evaluated an in vitro assay to analyze the DPPH scavenging activity. The DPPH solution was utilized in this experiment. In this activity, concentrations of 20, 40, 60, 80, and 100 µg were tested, and *S. virginianum* scavenging rates of 21.81±1.88 percent, 38.17±1.67 percent, 51.73±0.88 percent, 63.01±1.70 percent, and 71.01±0.43 percent were revealed, respectively. And *A. nilotica* rates of 25.81±1.49 percent, 40.77±1.80 percent, 54.43±0.70 percent, 65.81±1.39 percent, and 75.31±0.55 percent were discovered, respectively. The greatest DPPH scavenging recorded was 71.01 and 75.31 percent at 100 µg, and the maximum prevention of standard Ascorbic acid was 87±1.62 percent at a concentration of 100 µg/ml as shown in figure 3.

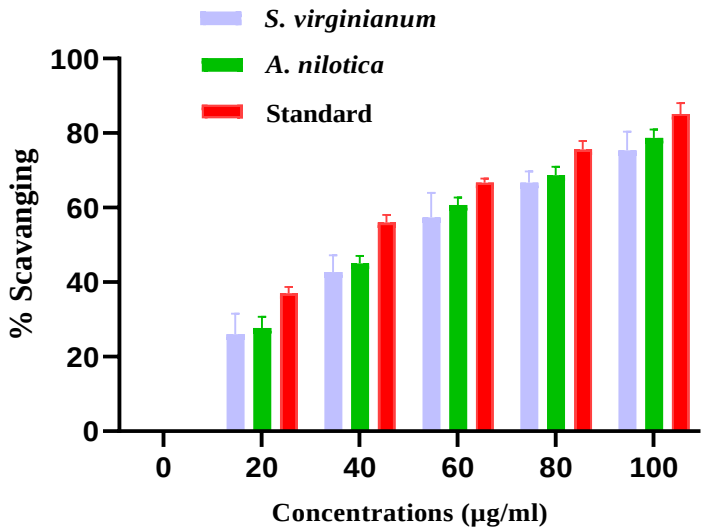

Figure.3 DPPH Free radical scavenging activity shown by the various doses of *S. virginianum*, *A. nilotica* and *ascorbic acid*

Discussion

Inflammation is the reaction of living tissues to injuries and infections. Chemicals and cells linked to inflammation are triggered or generated in large numbers in several situations that do not involve tissue damage or infection [20]. The development of novel, active and safer alternatives to NSAIDS is a main challenge for researchers. The anti-inflammatory drugs with great selectivity for the COX-2 enzyme are reflected to be safer as they are devoid of severe gastro-intestinal side effects [2]. Natural products from medicinal plants have been considered an alternative source of pharmacological molecules with negligible side effects [21].

S. virginianum and *A. nilotica* are common plant species. Phytochemical estimation of the various extracts of both plants discloses the presence of Diosgenin, β-sistosterol, carpesterol, and some others like Solasonine, Solamargine, B2-Solamargine, oleanolic acid, and caffeic acid compounds from *S. virginianum.* From *A. nilotica*, different alkaloids, sterols, saponins, glycosides and flavonoids have been reported [22]. The anti-inflammatory activities were investigated based on the folklore information using in vitro methods.

HRBC membrane stabilization and heat-induced hemolysis methods were selected for the in vitro assessment of anti-inflammatory potentials because the RBC membrane is like the lysosomal membrane, and its stabilization suggests that the fractions may as well stabilize lysosomal membranes [23]. Steadiness of the lysosomal membrane is important in reducing the inflammatory reaction by inhibiting the release of lysosomal components by stimulated neutrophils, which causes further inflammation and destruction. The results indicated that the extracts of *S. virginianum* and *A. nilotica* at various doses have significant anti-inflammatory potential. In analogy to the present study, in different studies [24], the ethanolic extract of *S. xanthocarpum* at 6mg/ml protected HRBC in hypotonic solution by 50%, the extract of *Acacia officinarum* at 6 mg/ml protected HRBC by 56%, and the combination of extracts (1:1 ratio) at 6 mg/ml protected HRBC by 67%. The results were surprising when all the data was compared to a normal standard drug, which had a protection level of 70% at a dosage of 2.5 mg/ml.

To fight free radicals, high concentration can cause damage to proteins and lipids and several other ailments, without any negative effects, many plant products have been discovered to obtain antioxidant [25]. Both extracts were used to determine the antioxidant activity of *S. virginianum* and *A.nilotica*. *S.virginianum* and *A. nilotica* greatest DPPH scavenging recorded were 71.01±0.43 and 75.31±0.55 percent at 100 µg, and the maximum prevention of standard Ascorbic acid was 87 percent at a concentration of 100 µg/ml.

The present results indicate the efficacy of *S. virginianum* and *A. nilotica* as efficient therapeutic agents in the treatment of inflammation. The results of this study authenticate the folklore knowledge of both plants. Further comprehensive studies are in progress for the isolation of the active components responsible for these potentials and the exploration of the possible mechanisms of their anti-inflammatory properties.

Conclusions

This study has confirmed the effectiveness and dominant anti-inflammatory and antioxidant potentials of the selected plants in different in vitro tests. These activities may be due to the presence of flavonoids. Thus, *S. virginianum* and *A. nilotica* with their potent anti-inflammatory and antioxidant effects, can be suggested as effective anti-inflammatory and

antioxidant natural Remedies. However, more investigation and detailed mechanistic studies are required to validate the current findings and explore the mechanism of action at the molecular level.

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