Phytochemical Screening and *In- vitro* Anti-inflammatory Activity of Stem Bark of *Samaneasaman*

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Introduction

Samaneasaman is tropical American origin tree belonging to the family Fabaceae. It is widely planted throughout the West Indies, Southern Mexico, Brazil and tropical region of the world and are found all over Pakistan (Ali, 1972). The tree was introduced to Ceylon in 1851 (Dassanayake, 1980). It usually reaches a height of 25m and has diameter of about 40m and is considered as a multipurpose tree (Ogunshe et al., 2006). The leaves, bark, root, seeds and pods of the tree are used in traditional system of medicine. The alcoholic extract of the leaves inhibited *Mycobacterium tuberculosis*, the alkaloid fraction of leaves is effective on the central nervous system and its infusion is used as laxative. Seeds are chewed for sore throat. A decoction of the inner bark and fresh leaves are used for the treatment of diarrhea, cold and intestinal ailments (Perry, 1980; Ayensu, 1981). In Jamaica the leaf is made into an infusion for treating blood pressure and the seeds are chewed in tropical Africa for treating inflammation of the gums and throat (Pius, 2011). *A.lebbeck* is a highly used medicinal plant in India among different traditional medical systems such as Ayurveda, Siddha and Unani (Kirtikar & Basu, 1981).In Sri Lanka *S. saman* is substituted to *Albizia lebbeck* in theAyurvedic raw drug market.. *A. lebbeck* is an ingredient of herbal preparations used for inflammatory joint diseases (Ayurveda Pharmacopiea, 1979). Because of the restricted distribution or rarity of *A. lebbeck*, *S. saman is* one plant that replaces as an alternative source for *A. lebbeck in* the ayurvedic drug market.

Objectives of the Study

Therefore, the present work was aimed to investigate the *in-vitro*- anti-inflammatory activity.

Phytochemicals are naturally occurring secondary metabolites produced in plants that help to provide their characteristic odor, flavor, smell, texture of plants and control diseases. The most important bioactive constituents present in plants are alkaloids, tannins, flavonoids, steroids, terpenoids, carbohydrate and phenolic compounds (Pascaline et al., 2011). Stabilization of lysosomal membrane is important in limiting the inflammatory response by inhibiting the release of lysosomal constituents of activated neutrophils such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extra cellular release or by stabilizing the lysosomal membrane. Human red blood cell (HRBC) membrane is analogous to the lysosomal membrane (Seema, 2011). Stabilization of HRBC membrane by hypotonicity induced membrane lysis can be taken as *in vitro* measure of anti- inflammatory activity of the drugs or plant extracts.

Methodology

Preparation of Solvent and Aqueous Extracts

Samples of *S. saman* was collected from their natural habitats and authenticated from National Herbarium. Stem bark of *S. saman* was shade dried and powdered using mechanical grinder (Disk mill, sttc 23, China), powdered stem bark (1kg) was subjected to sequential solvent extraction using hexane (2L), chloroform (2L), and methanol (2L) after keeping overnight in the shaker (orbital shaker, Lab-Line UK) separately. Each extract was filtered and the solvent was removed using rotary evaporator (Buchi R-114, Switzerland) and the yield of each extract was recorded.

Powdered stem bark (250g) was mixed with 750ml of distilled water and kept in the shaker overnight. The filtrate was freeze dried using the freeze dryer (Labconco,cat. 01,Missouri) and the weight of the crude was recorded.

Phytochemical Analysis

Stock solution of each extract (1%w/v) was prepared using an appropriate solvent (hexane or chloroform or methanol or water). The extracts obtained were subjected to preliminary phytochemical analysis using the methods described by Rajan et al. (2011).

In Vitroantiv-Inflammatory Activity

In vitro anti- inflammatory activity of *S. saman* extract was assessed by HRBC membrane stabilizing method (Anandarajagopal et. al., 2013; Anosike et al., 2012) with slight modification. Fresh blood (3ml) was collected from healthy volunteers into heparinized tubes that were centrifuged at 3000rpm for 10 min. A volume of normal saline equivalent to that of the supernatant was used to dissolve the red blood pellets. The volume of the dissolved red blood pellets obtained were measured and reconstituted as 40% v/v suspension with isotonic buffer solution (sodium phosphate buffer pH 7.4).

The reaction mixture (4-5ml) consisted 2ml of hypotonic saline (0.25% w/v NaCl), 1ml of phosphate buffer (pH 7.4), 0.1ml of HRBC suspension, 1ml of test solution and Diclofenac Sodium as the standard drug in DMSO (Dimethyl Sulphoxide) in different concentrations of (4,2,1,0.5, 0.25 mg/ml).For control distilled water (1ml) was taken instead of hypotonic saline and added 1ml of DMSO to the assay mixture. The assay mixtures were incubated at 56^0c for 30 min and cooled at running tap water, centrifuged at 3000 rpm for 15min. The absorbance of supernatant was read at 560nm using visible spectrophotometer. The percentage inhibition of hemolysis was calculated using the following formula.Inhibition of hemolysis =100*{Absorbance of control – Absorbance of test}/Absorbance of control

Key Findings

The preliminary phytochemical analysis of *S.saman* revealed that there was a presence of various secondary metabolites such as tannins and phenolic compounds saponins, steroids, flavonoids and glycosides in the methanol and water extracts (Table 1).

Table 1: Phytochemical Constituents of Methanolic Bark Extract of *S. Saman*

Phyto Constituents	Methanolic Extract	Aqueous Extract
Flavonoids	+	+
Alkaloids	+	-
Glycosides	+	-
Tannins and Phenolic	+	+
Saponin	-	+
Carbohydrates	+	-

Note: - = Negative (absent); + = Positive (present)

S.saman at different concentrations (4, 2, 1, 0.5, 0.25mg/ml) showed highest percentage inhibition of hemolysis 51 percent at its highest concentration when compared to the29 percent inhibition of hemolysis was shown by the standard drug Diclofenac Sodiumat its highest concentration (Figure 1 and 2). Non-steroidal anti-inflammatory drugs (NSAIDS s) exert their beneficial effects by either inhibiting the release of lysosomal enzymes or by stabilizing the lysosomal membrane (Mounnissamy et al., 2008). Exposure of red blood cells to injurious substances such as hypotonic medium, heat, methyl salicylate or phenylhydrazine results in the lyses of the membranes accompanied by haemolysis and oxidation of haemoglobin (Feirrali et al., 1992). Since human red blood cell membranes are similar to lysosomal membrane components (Mounnissamy et al., 2008), inhibition of cell membrane lyses was taken as a measure of the mechanism of anti-inflammatory activity of *S. saman* stem bark extract. Many reports have shown that plant flavonoids possess potant anti- inflammatory and anti-oxidant properties (Middleton 1992; Read 1995; Halliwel et al., 2005).Therefore presence of flavonoids, tannins and phenolic compounds and glycosides in this plant extract exhibited its anti- inflammatory activity.

Figure 1: *In Vitro* Anti-Inflammatory Activity of Methanolic Bark Extract of *S. Saman*

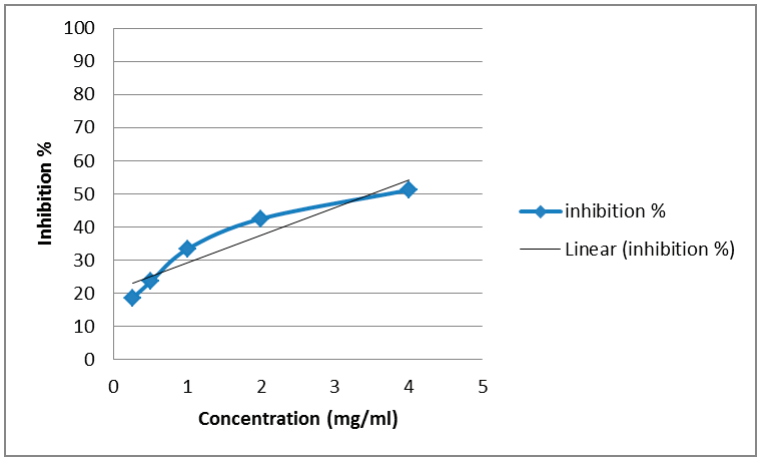
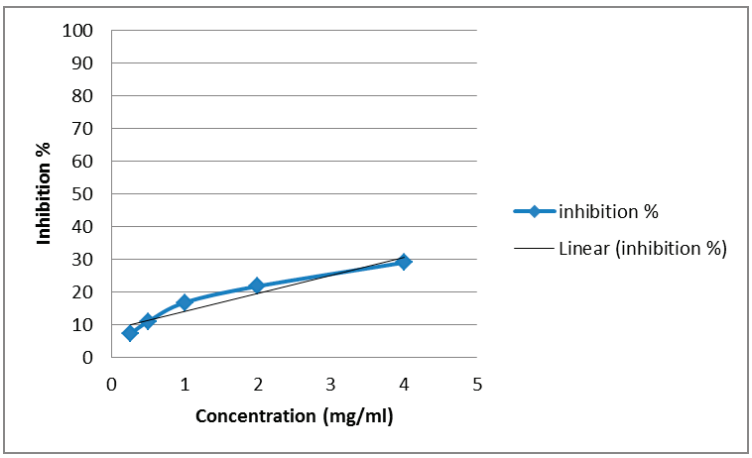


Figure 2: In *Vitro* Anti-Inflammatory Activity of Diclofenac Sodium



Conclusion

These findings provided useful information in identifying raw drug material for anti-inflammatory drug preparations and new drugs can be developed after investigation of experimental models and further isolation, purification, characterization and elucidation of the structure of the bioactive compounds of this plant species.

Keywords: Anti-Inflammatory; Medicnal Properties; Methanolic Extracts Samanea Saman; Phytochemicals

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