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Research Article

In Vitro Biological activity of Traditionally Used Wild Medicinal Plants of Solanaceae from Sibi District Balochistan Pakistan

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Abstract

Solanaceae plants have been identified as one of the main resource of traditional medicines and rich in bioactive compounds mainly in high valued Wild taxa. The present study is aimed to investigate the presence of phytochemicals and *in-vitro* anti diabetic and anti-inflammatory activity of six wild taxa belonging to three genera of Solanaceae frequently used by the indigenous communities of Sibi District, Balochistan Pakistan. Plant samples were collected during various field surveys in town and suburbs. Different plant parts were subjected to determine the phytochemicals, Anti-diabetic activity by using *in vitro* α-amylase enzyme inhibition method and anti-inflammatory activity by protein denaturation method. Results exhibited the presence of total phenol, flavonoid content, alkaloids, tannins and saponins in different parts of selected plants. Seeds of *Withania somnifera* exhibited the highest anti-inflammatory activity (84.5±0.5% Inhibition) followed by Seeds of *WC* (81.5±2%). Whereas, the highest anti-diabetic activity was depicted in seeds of *W. coagulans* (81. 5±0.5% Inh.) followed by followed by Seeds of *WC* (81.5±2%). Findings of the study revealed that a significant amount of biologically active components are present in selected plants. The outcome of the study will augment the better understanding about the potential use of these plants in nutraceutical and pharmaceutical formulations of selected plants. These plants can be utilized for new phytopharmaceutical and nutraceutical preparations. Conservation through cultivation of these significant medicinal taxa of the family is highly recommended.

Keyword: Anti-oxidant; Balochistan; Bioactivity; Phytochemicals; Sibi; Solanaceae

Introduction

Medicinal plants have long been used in traditional health care system to treat various ailments and their bioactive components are a significant resource of therapeutic development, mostly against many infectious diseases. Natural products derived from medicinal plants have gathered much attention in recent years as potential bioactive constituents. Wild Medicinal Plants (WMPs) play a vital role in our daily life and used in traditional medicine as well as recent drugs

discoveries. Some medicinal plants are augmented with diverse bioactive compounds that have been reported as beneficial in prevention and treatment of various ailments for maintaining a healthy life. These plants contain phytochemical constituents' viz., alkaloids, saponins polyphenols, flavonoids, terpenoids, and minerals etc. WMPs are rich source of essential and non-essential elements but excessive use of these elements may be harmful. According to World Health Organization, almost 80% world's populations depend upon traditional medicines for their health care system for attaining high-quality drugs [1], hence making them the reliable resource of curative healthcare products. Many potential drugs have been developed from bioactive constituents of medicinal plants. Crude extracts of medicinal plants are biologically more active than their isolated compounds because of their synergistic effects [2].

The Solanaceae is an economically important and one of the largest flowering plant families. It provides food and medicinal security around the globe. It comprises of approximately 2300 species and is described as significant sources of phytochemicals and dietary compounds in the pharmaceutical and food industry [3,4]. Some earlier reports on diverse taxa of family Solanaceae were comprised of phytochemical investigation of *Solanum incanum* [5]; basic phytochemical analysis and antimicrobial activity of *S. torvum* [6]; biochemical profiling, antioxidant and anti-inflammatory activities of *Physalis peruviana* [7] comparative structural, cytological and phytochemical investigation of *Capsicum* spp. [8] and biochemical, dietary and toxicological analyses of various plant parts of *S. macrocarpon* [9] have been described. *Solanum* genus has a number of species found in tropical and subtropical regions of the world. Many taxa are used in folk medicine and are used as food supplements. Amongst many species, *S. nigrum* has been considered as an important traditionally used plant in healthcare system for the treatment of many diseases. The genus *Withania* of solanaceae includes 24 species has been known for its high therapeutic and nutraceutical potential.

The genus *Physalis* of Solanaceae family comprises more than 90 species distributed around the globe in Pakistan the genus is represented by 3 species [10].

The study area Sibi has globally recorded as one of the high temperatures zone, with an average of 52 °C in summers. It is also known as one of the "Hot spot" regions of Pakistan. The majority of the population lives in rural areas and most of their resources are based on livestock, Agriculture, timber, food and medicinal plants etc. The indigenous people, including health practitioners are still relying on WMP's as a primary healthcare system. Hence, the study is aimed to evaluate the Traditional knowledge, phytochemicals and bioactive potential of some significant WMP's of Solanaceae from Sibi. The outcome of the study will augment the better understanding about the potential use of these plants in nutraceutical and pharmaceutical formulations of selected plants.

Materials and Methods

Plant collection

Six medicinal plants of three genera of family Solanaceae were collected from town and suburbs of Sibi, Balochistan Pakistan (Table 1). Samples were identified by Dr. Shazia Saeed and authenticated with the flora of Pakistan and POWO. The voucher specimens were submitted in Molecular systematics, biodiversity and conservation Lab, Department of Botany, University of Balochistan Quetta, Pakistan.

Table 1: List of plants with their accession no and site description

Plant Name	Plant code	Parts used traditionally	Accession No.	Site of collection	Elevation (meter above sea level)
Physalis divaricata	PD	L, S, R	QUETTA000259	Luni village	130-140
Solanum elaeagnifolium	SE	L, S	QUETTA000098	Nari Guage	200-400
S. nigrum	SN	S, L	QUETTA000009	Sibi city	130-150
S. virginianum	SV	L, R,S	QUETTA000150	Nari Guage	200-400
Withania coagulans	WC	L, S	QUETTA000018	Khaijak village	130-150
W. somnifera	WS	L, R, S	QUETTA000283	Nari Guage	200-400

L=Leaves, R=Root, S=Seeds,

Preparation of extracts

Collected plant samples of six plants were washed with distilled water to remove the contaminations. Different parts including leave, seed and root were separated and shade dried at room temperature. After that samples were ground by using pestle and mortar. Samples (50 g) required for each part of plant. Samples extract is prepared by following the method of [11].

Phytochemicals analysis

The Total Phenolic Content (TPC), Total Flavonoids Content (TFP), tannins, alkaloids and saponins from extracts were estimated using method of [12].

***In vitro* biological activities**

Anti-diabetic activity

The α-amylase enzyme inhibition method is used for estimating the *in vitro* anti-diabetic activity [13]. The absorbance was measured at 546 nm in a spectrophotometer. The % age α-amylase enzyme inhibitions in plants were calculated by using the formula:

$$\%\text{age inhibition of } \alpha\text{-amylase enzyme} = \frac{\text{Enzyme activity of control- Enzyme activity of extract}}{\text{Enzyme activity of control}} \times 100 = 1$$

Standard drug Metformin is used.

Anti-inflammatory activity

Anti-inflammatory activity was examined by using protein denaturation assay of [14]. Diclofenac sodium was used as a reference drug with the five concentrations i.e 100-500 μg/mL. The protein denaturation %age inhibition was calculated as:

$$\textbf{\% age inhibition} = \frac{\textbf{[Abs control-Abs sample]}}{\textbf{Abs control}} \textbf{ x 100 =2}$$

Standard drug Diclofenac sodium is used.

Statistical analysis

Data was recorded in mean ± SEM. Resulting data was analysed by applying descriptive statistics with three replications each by using minitab software.

Results and Discussion

Six plants including three genera of family Solanaceae from Sibi District were selected for the phytochemical investigation. These plants are abundantly used by folks in traditional medicines as therapeutic drugs.

Physalis divaricate

The plant is collected from village named Luni, Sibi District Balochistan Pakistan. Its plane area elevation is approximately 132 masl. The plant is a valuable resource and utilized as dietary supplement as well as medicinally important. Traditionally plant is reported to treat Hepatitis, jaundice and liver problems from the study area. Earlier plant is also reported in Iranian traditional health care system, the taxon has primarily been used to cure respiratory and hepatic disorders and

excretory system insufficiency. The seed extract of *Physalis* species has also been found to have beneficial effects against jaundice [15]. In Brazil and Amazon rainforest of Peru indigenous people also utilize *Physalis angulata* for hepatic disorders [16]. In India and China the root extract of *P. divaricata* is extensively used to cure hepatic disorders by the indigenous tribes (Bhoxa Community) and by the Maona tribes respectively [17,18].

Preliminary phytochemical screening of different parts of plant of area exhibited the presence of TPC, TFC, alkaloids, saponins and tannin (Table 2). Earlier some elements from *P. divaricata* were reported from Swabi Pakistan [19]. Medicinally important plant is used to treat different ailments as analgesic, antiseptic, asthma, diarrhea, treatment of malaria, liver disorders, Kidney problem, Anti-cancer, diuretic, rheumatism, indigestion [19-21].

Solanum elaeagnifolium

The plant is collected from village named Nari Guage, Sibi District Balochistan Pakistan. Nari Guage is comparatively high altitudinal zone in the District Sibi approximately 200-450 masl. Plant is a deep-rooted summer-growing perennial undershrub. *S. elaeagnifolium* is called silver leaf nightshade and locally by the pashtoon tribes of the area, the taxon is termed as “Shin Gul”means blue or violet flowered plant. Traditionally plant is used for the treatment of sore throats as an antiseptic agent, toothaches, and gastrointestinal disorders. Recently it has been depicted that *S. elaegnifolium* is invading the agricultural fields as a weed, besides its traditional significance the taxon may be restricted to some arid regions to protect economically important crops of the region in general and overall of the province in particular. Phytochemical screening results reveal the presence of TPC, TFC, alkaloids, saponins and tannin in different parts of plant of area (Table 2). Plant is used to treat different ailments in traditional medicines. The fresh or dried root is used sucking snakebite, or other poisonous insects and the crushed root used as a plaster. Also used as insecticidal for different crops. Earlier reports also found its medicinal importance like laxative, antioxidant activities sore throats, an antiseptic agent, toothaches, and gastrointestinal disorders [22-25].

S. nigrum

The plant is collected from Sibi town and suburbs. The taxon is commonly found as a weed in moist habitats in different kinds of soils. Due to its great medicinal importance different phytochemicals were investigated from this specie. Traditionally plant is used to treat fever, cough and indigestion, ulcers and skin diseases. It is also an economically important plant used as Wild Food Plant in Sibi and other parts of Balochistan, Pakistan by various tribal communities. Ripened berries and boiled, leaves are edible and either consumed raw or cooked as vegetable. In present study phytochemical screening of different parts of plant of area showed the presence of TPC, TFC, alkaloids, saponins and tannin (Table 2). Earlier *S. nigrum* have exhibited many pharmacological properties including pneumonia aching teeth, stomach ache, tonsillitis, wing worms, pain, inflammation, fever, tumour, as tonic, as antioxidant, as anti-inflammatory, as hepatprotective, as diuretic, and as antipyretic, antitumor, immunomodulatory, antihypertensive, antimicrobial, and antiviral activities [26-28].

S. virginianum

The plant is collected from village named Nari Guage, Sibi District Balochistan Pakistan. It is an annual herbaceous plant grown in wild. It is traditionally used to treat asthma, skin diseases, allergy, cough, fever. Earlier reported from different mountainous range of Balochistan [29]. Preliminary phytochemical screening of different parts of

plant of area showed the presence of TPC, TFC, alkaloids, saponins and tannin (Table 2). Earlier reports found that plant is used as antifungal agent, insecticidal, antihelminthic, and anti-inflammatory agents, antibacterial, antimicrobial, antitumor, antipyretic and analgesic activities **[30-32]**.

Withania coagulans

One of the plants of genus Withania, *W. coagulans* is collected from village Khaijak of Sibi District ,elevation range approximately 130 to 150 masl, traditional uses of the plant is earlier reported from different regions of Balochistan **[29,33]**. Preliminary phytochemical assay of different parts of plant collected from the study sites exhibited the presence of TPC, TFC, alkaloids, saponins and tannin (Table 2). Earlier reports assessed that this valuable medicinal plant has been used to treat various ailments including; abnormal cell propagation, wasting and neural disorders, glycaemia, insomnia and hepatic disorders. Due to the presence of an active compound withanolides, various human health related problems can be resolved **[34,35]**.

W. somnifera

another taxon studied in the present study is *W. somnifera*, collected from village named Khaijak of the District Sibi. It possessed a large number folk medicinal uses and pharmacological applications, locally known as "Lakri" and "Asghand" in the study area by different tribes. Preliminary phytochemical screening of different parts of plant of area showed the presence of TPC, TFC, alkaloids, saponins and tannin. TPC were higher in seeds extract followed by leaves and roots (Table 2). Previous studies reported that plant is significantly used to treat tumors, inflammation, apoptosis angiogenesis, and stress **[36-38]**.

Table 2: Phytochemical screening of six plants of family Solanaceae from Sibi district

Plant Code	TPC	TFC	Alkaloids	Tannins	Saponins
PD (Seed)	+	+	+	+	+
PD (Leaf)	+	+	+	+	+
PD (Root)	+	+	+	+	+
SE (Seed)	+	+	+	+	+
SE (Leaf)	+	+	+	+	+
SE (Root)	+	+	+	+	+
SN (Seed)	+	+	+	+	+
SN (Leaf)	+	+	+	+	+
SN (Root)	+	+	+	+	+
SV (Seed)	+	+	+	+	+
SV (Leaf)	+	+	+	+	+
SV (Root)	+	+	+	+	+
WC (Seed)	+	+	+	+	+
WC (Leaf)	+	+	+	+	+
WC (Root)	+	+	+	+	+
WS (Seed)	+	+	+	+	+
WS (Leaf)	+	+	+	+	+
WS (Root)	+	+	+	+	+

Presence=(+), Absent=(-)

In-vitro Biological activity

Anti-inflammatory

The presence of phytochemicals reveals the significant inhibitory effect among different parts of all six selected plants of Solanaceae on albumin denaturation when compared with the standard drug (Fig. 1). Significant inhibition of albumin was observed among different taxa. *WS* seeds depicted maximum inhibition with the highest value (84.5±0.5% Inh.) followed by Seeds of *WC* (81.5±2%).

Anti-inflammatory activity of PD showed in seed (67.5±0.5% Inh.), leaf (46±1% Inh.) and root (47.5±0.5% Inh.). SE exhibited in seed (64.5±1.5% Inh.), leaf 30.5±0.5% Inh.) and root (37±1% Inh.). SN exhibited in seed (71.5±0.5% Inh.), leaf (56±1% Inh.) and root (62.5±0.5% Inh.). SV in seed exhibited (43±2% Inh.), leaf (36±2% Inh.) and root (36.5±1.5% Inh.). WC exhibited in seed (81.5±0.5% Inh.), leaf (67.5±1% Inh.) and root (70±1% Inh.). WS exhibited in seed (84.5±0.5% Inh.), leaf (69.5±0.5% Inh.) and root (69.5±1.5% Inh.).

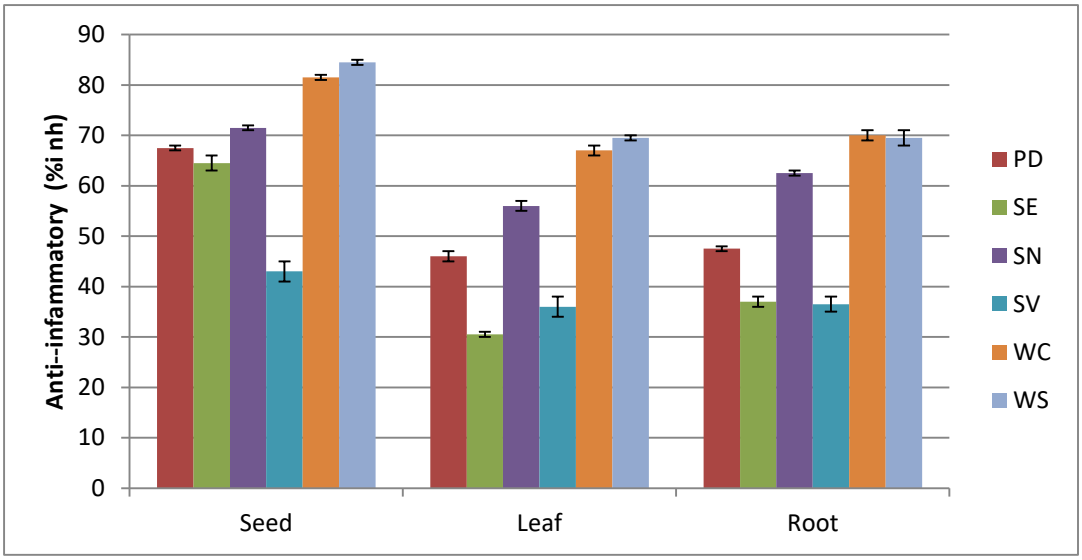

Figure 1: Comparison of Anti-inflammatory activity among different parts and taxa of Solanaceae (Listed in Table 1)

Anti-diabetic activity

Different parts of all six selected plants revealed significant differences in anti-diabetic activity when compared with the standard drug Metformin (Fig. 2). The seeds of WC (81. 5±0.5% Inh.) exhibited the highest anti-diabetic activity among all selected species. It was followed by seeds of WS (80.5±0.5%).

Anti-diabetic activity of PD exhibited in seed (54.5±0.5% Inh.), leaf (34±1% Inh.) and root (36.5±0.5% Inh.). SE exhibited in seed (63±2% Inh.), leaf 44.5±0.5% Inh.) and root (41.5±1.5% Inh.). SN exhibited in seed (66.5±0.5% Inh.), leaf 43.5±0.5% Inh.) and root (40.5±0.5% Inh.). SV in seed exhibited (71.5±2.5% Inh.), leaf 36±2% Inh.) and root (41.5±0.5% Inh.). WC exhibited in seed (81.5±0.5% Inh.), leaf 66.5±1.5% Inh.) and root (47.5±0.5% Inh.). WS exhibited in seed (80.5±0.5% Inh.), leaf 67±1% Inh.) and root (40±1% Inh.).

Earlier reports were in agreement with our studies ethnobotanical studies and traditional use of plant to treat diabetes and inflammation is being in practice around the world [29, 39-42].

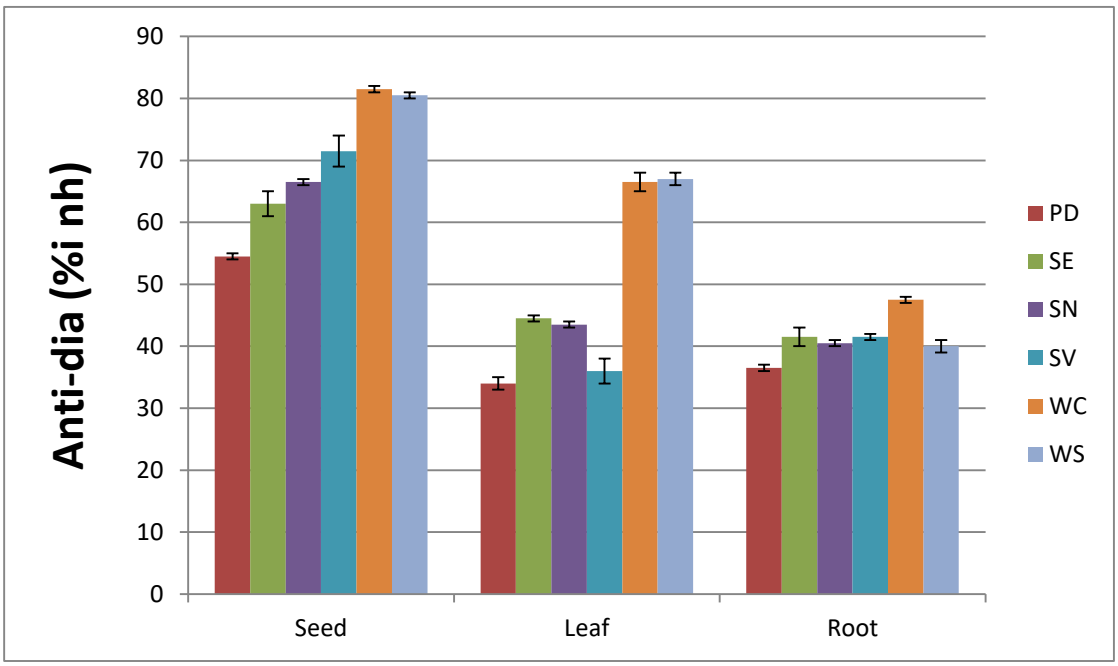

Figure 2: Comparison of Anti-diabetic activity of different taxa of Solanaceae (Listed in Table 1)

Conclusion

The current study showed the presence of phytochemicals in all six selected plants of Solanaceae. These plants are in use of local communities of Sibi District, Balochistan Pakistan. Present study validated the use of different parts of these plants for treating different ailments. The method of these plant uses with different doses need to be validated for authentication. Furthermore, biological activity (*in vivo*) is highly recommended. The conservation through cultivation of these significant medicinal taxa of the family is also need attention so that these plants should be incorporated for health care system.

Authors' contributions

Conceived and designed the experiments: S Saeed, Performed the experiments: B Maria, Analyzed the data: B Maria & A Ahmed, Contributed materials/ analysis/ tools: S Begum & M Ahmed, Wrote the paper: A Ahmed.

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