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EVALUATION OF BIOACTIVE POTENTIAL OF *DALBERGIA SISSOO* (*ROXB.*) AND *CARALLUMA TUBERCULATA* (N.E.BR.) AND THEIR TOXICOLOGICAL ASSESSMENT.

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Abstract
Dalbergia sisso (Roxb.) and *Caralluma tuberculata* (N.E.Br.) are extremely useful medicinal plant species distributed in various parts of the world including Pakistan. Traditionally Pakistani people use these plants to treat Diabetes mellitus, arthritis, obesity, and certain cardiovascular problems. Folks are using these medicinal plants abundantly. There is a dire need to perform scientific screening of these valuable plants. In order to establish evidence based resource regarding the ethnobotanical use of these herbs present studies were carried out. The diverse phytopharmacological activities comprising of in-vitro antidiabetic, antioxidant, anticancer, phytotoxic, insecticidal and brine shrimp activities were carried out. Promising antioxidant and in vitro antidiabetic actions were explored. Both species were found safe and effective with considerable anti- oxidant and antidiabetic actions. Further in depth in vivo research is required to validate the safe and effective use of these valuable traditional herbal therapeutic alternatives.

Keywords: Pharmacological, safety, Antioxidant, Antidiabetic, Herbal, Insecticidal, Phytotoxic, Anticancer, Diabetes mellitus. *Dalbergia sissoo*, *Caralluma tuberculata* , medicinal plants.

INTRODUCTION

In Phytotherapy medicinal plants and their extracts are used as therapeutic agent. It is also termed as folk medicine, botanical medicine, medical Herbalism, herbal medicines natural medicines and herbology (Hatami et al., 2024). Medicinal plants, also called medicinal herbs, have also been practiced in traditional medicine practices since prehistoric times. *Dalbergia sisso* (Roxb.) is known to have timber value, which is being used in wood works, furniture and frame work. Over the past few years the research has started for exploration of its medicinal significance. It is useful for controlling the hyperlipidemia effect of the cholesterol in the blood (Khera & Bhatia, 2014).

*Caralluma tuberculata (N. E. Brown)*is a succulent, perennial herb growing in the wild in Pakistan, India , United Arab Emirates, Saudi Arabia, south east of Egypt, Iran and Nigeria (Islam et al., 2015). It is commonly found in different areas of Pakistan such as in Punjab, Khyber Pakhtoonkhuwa, (Waziristan, Dir,) and Baluchistan (Nimargh, Harboi, Nichara, Gidar),provinces of Pakistan (Adnan et al., 2014) Number of biological active and important chemical constituents have been studied from thisplant. Important constituents include Caratuberside A, B, C, D, A2, E and F (Cao et al., 2019).

Presence of bioactive constituents have resultedinto various pharmacological actions in medicinal plants Using other methods including gamma rays, X-rays, and genome editing tools, researchers have created a large number of Lesions mimic mutants (LMMs) in plants (Haroon et al., 2023). Pharmacological actions, if any, of manyplants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety. Antidiabetic and antioxidant actions of medicinal plants are ofteninvestigated in order to establish the medicinal potential of a medicinal plant. Diabetes mellitus is most common endocrine disorder. According toworld health organization (2014) global prevalenceof diabetes was estimated to 9% among adults. Prevalence of disease will grow to 366 million people till 2030, showing an increase of 144%. Mortality rate related to diabetes is about 9% globally. Healthcare cost of diabetes varies between 2.5% - 15% depending on the prevalence and treatments available locally. Diabetes mellitusis a syndrome rather than a disease, referring to a collection of diseases associated with metabolic disorder characterized by a chronic hyper- glycemia with abnormal carbohydrates, proteinsand fat metabolism (Bennett & Brown, 2007). Itscomplications include cellular and tissue injury,resulting to end organ damage, if untreated.Severe complications including retinopathy, nephropathy, neuropathy and micro angiopathy can be avoided by a two way approach i.e. controlling the plasma glucose levels(according to American Diabetes Association Pre-prandial plasma glucose level should be between 90-130 m/dl and peak post prandial level should be <180mg/dl. ADA 2006) and protecting the cellsand tissues from injuries. Alpha glycosidaseenzyme, present in small intestine is responsiblefor breakdown of large, complex carbohydrate molecules to small simple and absorbable monosaccharides. Hence agents inhibiting this enzyme can be helpful for controlling glucose levels in plasma. Currently available treatment options for diabetes like oral hypoglycemic agents and insulin have their own limitations; hence there is a need for exploration of alternative treatment options. Many herbal medicines are recommended for the treatment of Diabetes mellitus (Gupta, 1994) and peptic ulcer (Mubashir et al., 2022). Thehypoglycemic effect of many herbs has alreadybeen reviewed. Antioxidants are used as supportive therapy in the treatment of DM (Garg et al., 2018). Hence the present studywas carried out to evaluate the Antidiabeticand antioxidant activities of plant selected plantsalong with several other investigations includingAntileishmanial, anticancer, insecticidal, Phytotoxic Lemna minor and Brine Shrimp. It's critical to employ additional strategies to manage stomach parasites. Using medicinal herbs with anthelmintic properties is one of the suggested populations. The custom of passing down knowledge about the application of medicinal plants from one generation to the next (Akram et al., 2023).

The objective of this study was to evaluate hypoglycemic and antioxidant, anticancer, Antileishmanial, Phytotoxic, insecticidal, Brine shrimp lethality, potential of pods, leaves and stem. Bark of Plant A and B. Methanolic extracts were used for this purpose. 2, 2-diphenyl -2-picrylhydrazyl (DPPH) free radical scavenging assay activities were performed for determination of antioxidant potential, and hypoglycemic potentials were evaluated by using antiglycation assay activities.

Fg-1:*Dalbergia sisso* Leaves and Pods

F ig-2: *Caralluma tuberculata* (N .E.Br.)

Methodology:

Chemicals used in these experimentations include analytical grade methanol (Merck Germany),2,2- Diphenyl-1-picrylhydazyl(DPPH),Bovine Serum Albumin (10mg/ml, Magnesium Oxide (MgO) 14mg, Phosphate Buffer 0.1 M , (pH 7.4), NaN3(3 m M)

Plant material Leaves, pods, and stem bark (5 Kg) each, of D. Sissoo(Roxb) were collected similarly 2 Kg vegetative parts of *Caralluma tuberculata* were collected, Voucher specimens were obtained from Herbarium aftersubmission of collected plant materials. After collection of plant materials

Methanolic extracts were prepared by using standard percolation method. Shortly Leaves, barks and pods of *Dalbergia sisso* were cleaned garbled chopped, and soaked in separate glass jars in methanol. Second plant *C.tuberculata* was also cleaned, garbled and chopped simultaneously. After two weeks soaked materials were filtered with Whitman filter paper no.1, and evaporated in rotary evaporator it was run at 42 ▫C to preserveheat sensitive constituents. Individual crude extracts were obtained one by one and stored inamber fluted glass containers, to avoid damage ofphotosensitive constituents. Methanolic extractswere weighed and transferred to separate testtubes for further studies.

The in vitro antidiabetic studies were performed using a method described by Lee et al., with slight modification((Lee et al., 1998), (Rahbar & Figarola, 2003)). Triplicate samples of BSA (10mg/mL) , 14mM MgO, and 0.1 M phosphate buffer(pH 7.4), containing NaN3 (3 mM) , incubated in wellplate containing 50µL BSA solution ,50µL MgO and 20µL test sample) at 37 ºC . After 9 days of Incubation, glycation of protein was monitored by measuring the specific fluorescence (Excitation, 330nm; Emission, 420nm) against blank through a microtiter plate spectrophotometer (Spectra Max, Molecular Devices CA, USA0. Rutin was used as a positive control.

The evaluation of the antioxidant capacity of different samples involves the use ofthe free radical, 2, 2-Dipehnyl-1-picylhydrazyl (DPPH). It is a rapid, simple and inexpensive method and is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of different samples by utilizing the stable 2, 2-diphenyl-1-picrylhydazyl (DPPH) radical .Theodd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The color turns from purple to paleyellow as the molar absorptivity of DPPH radicalat 515nm reduces when the odd electron ofDPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourization is stoichiometric with respect tonumber of electron captured. The 300 µ M solutionof 2, 2-diphenyl-1-picrylhydrazyl (DPPH) is prepared by dissolving it in ethanol (100%).Thetest samples were dissolved in DMSO (Dimethylsulfoxide .100%)To the 96 – Well Plate ,5 µL ofsample was added and pre reading was taken at 515 nm95 µL of DPPH was added to each well. The 96-Well plate was then incubated for 30minutes at 37 ÚC after covering with Para film toavoid evaporation of the solvent. Final absorbance was then recorded on micro plate reader at 515nm. Control contained only DMSO (100%).Gallicacid and N-acetyl cysteine is used as standard.The Percent radical scavenging activity wascalculated by using following equation: RSA (%) = 100- (O.D of sample/ O.D ofcontrol×100) (Kumari & Kakkar, 2008).

Antileishmanial evaluations were performed to assess the potential of this part of plant as Anti-Leishmanial agent. Incubation period was 72 h Incubation temperature was 22ºC.

Insecticidal evaluations were also conducted in order to discover using 1019.10 µg /Cm^{2}and permethrine was used as standard drug 239.5 µg /Cm^2 assessment was done by usingcontact toxicity method. Concentrations of sample used were1019.10 µg /Cm^{2}and permethrine drug 239.5 µg /Cm^2 was used as standard drug. Concentration of standardassessment was done by using contact toxicity method. Phytotoxicity studies were performed by modified protocol of Mc Laughlin et al (1991). Paraquat (conc) was used as standard drug. Incubation temperature was 28ºC with ±1ºC.Sample showed no activity. Brine shrimp assessment was performed by incubation of shrimps and testing the efficacy of extracts by application. Probability value 0.15 no of replica 3 Upper limit 79.2239, Lower limit 16.4201. Incubation conditions 28º±1ºC. Both showed positive lethality with LD_{50}, 40.0134 µ/ml (Sarah et al., 2017).

RESULTS:

Table 1. Antidiabetic E valuations of *D.sissoo* P od and Leaves and Bark

Sample code	**Conc.(m M)**	**% Inhibition**	**IC50 ±SEM[µM]**
Sisso Pod Extract	2mg	73	
	1mg	54	0.88± 0.6mgg/m l
	o.5m g	35	
Sisso Leave Extract	2	68	
	1	47	1.13± 1.12 m/m l
	0.5	31	
Sisso Bark Extract	0.5m g/ ml	-ve	Inactive
Rutin	2mg/ ml	95	0.176± 0.01mg/m l

Table -2 Antioxidant activity of *D.sissoo* Leaves, Bark and Pods:

Sample code	IC_{50} ± SEM µM	% RSA (Radical scavenging activity)
Sisso Leave extract	47.47±0.69	75.09
Sisso Bark extract		76.09
Sisso Pod Extract		80.90
Gallic acid	23.436±0.43	93.93
N-acetyl cysteine	111.44±0-7	95.95

Table -3 Anti-cancer Evaluation of *Dalbergia sissoo* (Roxb.) leaves.

Sample code	Concentration (mg/ml)	Inhibition (%)	IC_{50} + SD
Sisso Leave Extract	50	24.62	50µ M
Doxorubicin	50	89.29	0.92±0.1

Table-4 Anticancer Evaluation of *Caralluma tuberculata* N.E.Br

Sample code	Concentration (mg/ml)	Inhibition (%)	IC_{50} + SD
Sisso Leave Extract	30	26.01	50µ M
Doxorubicin	30	80.4	0.21±0.1

Table - 5 Antileishmanial Evaluation of *Caralluma tuberculata*

Leishmanial Activity		IC 50 (µg/ml)± SD
Test compound Caralluma extract		>100
Standard Drugs	Amphotericin B	0.29±0.05
	Pentamidine	5.09±0-09

Table -6 Insecticidal Activity of Caralluma tuberculata (CT) and *Dalbergia sisso* pods and leaves (DSP).

Name of Insect	Mortality (%) +ve control	-ve control	Sample Caralluma	SampleD Sisso Pod	SampleD Sisso Leaves
Tribolium castanium	100	0	0	0	0
Sitophilus oryzae	-	-	-	-	
Sitophilus Dominica	100	0	0	0	

Table -7 Phytotoxic Evaluations of *Dalbergia sissoo* Bark and Pods.

Name Of Plant	Conc.of Compound(Mg/ml)	No. of fronds			% Growth Regulation	Conc. Of standard drug µg /ml
		Sample Bark	Sample Pods	Control		
Lemna Minor	10	20	20	20	0%	0.015
	100	20	20		0 %	
	100	19	20		0%	

Table -8 Brine Shrimp Activity of Caralluma Extract:

Dose (µg/ml)	No. of Shrimps	No. of Survivors	LD_{50} µg/ml	Std.Drug	LD 50 µg/ml
10	30	20	440.014	Etoposide	7.4625
100	30	14			
1000	30	1			

DISCUSSION AND CONCLUSION:

Use of plants and plant based therapeutics has increased dramatically in recent decades. This trend shift is due reconsideration of mankind towards nature. A number of disease termed as lifestyle diseases including diabetes, cancer and degenerative diseases are clearly related to deviation from nature and natural life style. Sedentary life style and unhealthy eating habits can result into a collection of diseases or syndrome i.e. diabetes mellitus, hypertension, hypercholesterolemia and musculoskeletal problems. If untreated it may cause extremely harmful complications including vasculature changes (Laakso, 1999). Damages are caused by enhanced free radical generation. Suitable antioxidants are beneficial to minimize these damages due to the presence of compounds which are generally synthesized for their self-protection. Hence toxicological evaluation of crude extracts of plants is also needed. It's critical to employ additional strategies to manage stomach parasites. Using medicinal herbs with anthelmintic properties is one of the suggested populations. The custom of passing down knowledge about the application of medicinal plants from one generation to the next (Degla et al., 2022).

Methanolic extract of bark was applied 0.5mg/ml and found to be inactive against standard drug Acarbose which showed 50% inhibition at concentration 1mg/ml, whereas methanolic extract of pods showed dose dependents antidiabetic effect. It showed 35%, 54% and 73% inhibition at 0.5mg/ml, 1mg/ml and 2mg/ml concentration respectively. Similarly methanolic extract of leaves also showed significant antidiabetic effect. Its inhibitions were 31%, 47% and 68% at 0.5mg/ml, 1mg/ml and 2mg/ml concentrations respectively. Rutin was used as standard drug which showed 95% inhibition at 2 mg/ml. DSP was also tested for its antioxidant potential. This testing was conducted through RSA (Radical Scavenging Activity), Gallic acid and N-acetyl cysteine was used as standards. It showed 80.904% activity at IC_{50} 47.47 ± 0.69 µM. whereas Gallic acid sowed 93.93% and N-Acetyl cysteine showed 95.95% activity at IC_{50} 23.436±0.43 and 111.44 ± 0.7 respectively. Methanolic extracts of leaves and bark were also tested for antioxidant potential. Leaves showed 75.09% and barks showed 76.09 % free radical scavenging activity other experimental conditions were kept same as pods. Anticancer potential of methanolic extract of leaves was also tested against H-460 cells and Doxorubicin was used as standard drug. It showed 24.62 % inhibition at 50µg/ml concentration against Doxorubicin which showed 89.29 % inhibition in anticancer assay. DSL showed insignificant anticancer activity. Methanolic extracts of leave and pods were undertaken to determine their toxicological profile through performing Brine shrimp bioassay, Insecticidal and Phytotoxic profiles. In case of leaves 10,100 and 1000 µg/ml doses were applied 20 , 14 and 1 out 30 shrimps were survived at Lethal Dose. 440.014 µg/ml etoposide was used as standard drug at LD_{50} 7.4626 µg/ml which showed that leave extract is safe and can be used for medicinal purposes (Ahn, 2017). In case of pods number of survivors were 21, 20 and 1 out of 30 at 10,100 and 1000 µg/ml concentrations indicating the safety profile of methanolic extracts of pods. In insecticidal evaluations four insect species were used for insecticidal evaluations .*Tribolium castaneum, Sitophilus oryzae, Sitophilus dominica and Callosobruchus analis* were used as test animals. Pods (DSP), Leaves (DSL) and Bark (DSB) extracts showed 0% insecticidal activity hence safety profile was confirmed (Meshram *et al.*, 2000). Leaves extract was also tested against *Tribolium castaneum, Sitophilus oryzae, Sitophilus dominica and Callosobruchus analis* (Meshram, 2000).

Pods and leaves extracts were also tested against *Lemna minor* in phytotoxic activity profile and found to be safe. The vegetative part of *C.tuberculata* (N.E.Br.) was tested for its anticancer potential it showed 26.01% inhibition at 30 µg/ml concentration. Doxorubicin was used as standard drug for this testing, plant showed minimal anticancer effects against H-460 cell lines hence it may not be a good candidate as an anticancer lead (Hirono & Grasso, 1981). Antileishmanial evaluation was performed for plant B, Amphotericin B and Pentamidine were used as standard drug. Least significant Antileishmanial activity was observed therefore it can be used as Antileishmanial agent (Brenzan et al., 2007). *Tribolium castaneum, Sitophilus oryzae, Sitophilus dominica and Callosobruchus analis* were used for insecticidal evaluation of *C.tuberculata*. Methanolic extract of plant B showed minimal insecticidal action. The results confirmed the safety profile of the plant extract

(Meshram, 2000). Extracts were also tested for its Phytotoxicity *Lemna minor* was used as test organism results indicated that of plant extract was found to be safe (Wang & Williams, 1990). Plant breeding has been significantly impacted by NGS and is anticipated to remain so in the future. All things considered, NGS has enormous potential in plant breeding and genetics, and its application is probably only going to increase over the next few years (Ali et al., 2023).

Both plants exhibited variety of pharmacologicalactivities especially plant A showed Antidiabeticand antioxidant activity which are very valuabletherapeutically. Toxicological evaluations of bothplants confirmed the safety of these plants. Hence pharmacological investigations of these two important plants have proved to be very beneficial and serve as guide for future exploration of these plants as well as their pharmacological and toxicological profiles provedto be a good candidate of remedial agents in wide variety of disease conditions. Seven different types of Pharmacological assessmentswere conducted on various fractions of selectedplant species. Most significant results wereobtained by Antidiabetic and Antioxidant assessments. These results points towards further explorations of active fractions to serve as lead for development of new Antidiabetic and antioxidant agents. These findings also justifythe folkloric use of these herbs as Antidiabetic agents. Phytotoxic effects of these two plants also give clear indication of safety profile of these two medicinally important plant species. Anyhow brine shrimp lethality testing indicated the somewhat lethality of these plants. CRISPR/Cas technology potential has been described as modifying plant genomes for high yields under various biotic/abiotic stresses (Rehman et al., 2022).

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