Antidiabetic, anti-inflammatory and antioxidant activity of *Artemisia Roxburghiana* along with its qualitative and quantitative phytochemical Screening

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ABSTRACT

Objective

The objective of study was to conduct different assays for exploring pharmacological properties of medicinal plant Artemisia roxburghiana along with its phytochemical screening.

Methodology

Qualitative and quantitative tests phytochemical were carried out using different methods for total phenolic and flavonoid contents. Antioxidants capacity, total reducing power assay (TRP) and total antioxidant capacity (TAC) were determined. Formalin induced right hand paw edema method was used for investigation of anti-inflammatory activity. The anti-diabetic study was done by both in-vitro (α amylase inhibition assay) and in-vivo (Alloxan induced anti-diabetic study).

Results

The plant revealed the presence of active primary and secondary phytochemicals and exhibits significant anti-oxidant, anti-diabetic and anti-inflammatory activities. Radial scavenging activity (2, 2'-Diphenyl Picrylhydrazyl radical scavenging activity), total antioxidant capacity (TAC), and total reducing power (TRP) of *Artemisia roxburghiana* extracts at final concentration of 400 µg/mL is considerably significant. The presence of alkaloids, phenolic, flavonoids, terpenoids, reducing sugars, glycosides, steroids, carbohydrates and steroids was seen in all the extracts as well as effects of amylase etc.

Conclusion

This plant is a source of valuable natural products for pharmaceutical industries and can be used for identification and isolation of novel compounds which leads toward drug discovery for treatment of more serious and complicated diseases.

Key words

Artemisia, Antidiabetic, 2, 2'-Diphenyl Picrylhydrazyl, Anti-inflammatory, total antioxidant capacity, Alloxan, Formalin

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INTRODUCTION

There is a gradual and significant interest in medicinal plant and herbal materials due to the presence of natural antioxidants like flavonoids and phenolic plant extracts. Hence the improvement and development of a sophisticated standard techniques for efficient and fast extraction of these secondary substances remains a question.[1]

Medicinal herbs can be distinguished as the plants that have fruitful pharmacological effects on animal or human body and has beneficial properties.

Medicinal herbs have been employed against different diseases since long.

Therapeutic plants used in the therapy of basic disorders, for instant, cholera, tuberculosis, malaria, pneumonia and asthma etc. The usage of plants to cure few kinds of human diseases has a long history. Various parts of plants, for example, roots, stem, bark, and leaf are being used to stop, ease or revert symptoms of disease back to normal. Herbs have the most various great exploration and are the most secure commitment among home grown medications.

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Conventional medication is still accepted as the primary health care method in many rural communities, due to a number of reasons including potency, advantage and affordability.[2]

Medicinal plants are reservoir of natural products and are being used for treating different diseases since ancient times. Natural products procured from medicinal plants are main source of preventing and curing diseases.[3] According to World Health Organization (WHO) traditional medicines (TM) are widely used healthcare system and about 70-95% population relies upon herbal medicine for treatment of different diseases around the world in developing as well as in developed counties. Almost 80% population in Africa, 48% in Australia, 70% in Canada, 42% in USA, 38% in Belgium, 40% in China, and 75% in France relies on TM.[4]

Inflammation is an intricate biological response of vascular tissue to external stimuli pathogens and irritants identified by redness, swelling and pain. It is a worldwide problem and can be of both types either acute or chronic inflammation There is a need of more safe and potent way for treatment of inflammation. Many medicinal plants have anti-inflammatory activity and are used for producing new drugs that can be used for treatment of chronic and infectious diseases.[5]

Artemisia is one of the largest genera of the family Asteraceae and is included in the group Anthemideae. The genus of small herbs and shrubs comprising of over 500 species.[6] The genus was originated from semi-arid Asia and spread from central Asia to around the world.[7] *Artemisia roxburghiana* (*A. rox*) is one of the important medicinal specie of this group. An immortal herb of Himalayan zone between 1000 and 4300 m altitudes. It grows on dry slopes and wasteland with 50-100 cm height. It is identified by its hairy crawling roots, simple stem, leaves without hairs and purple color flowers.[8] Locally it is distributed in Islamabad, Rawalpindi, Swat and Kurram Agency at altitude of 2500 to 3000 m. Other than Pakistan, it is found in India, Nepal, Afghanistan, China, and Thailand. The ethnobotanical uses of the plants are that *A. rox* is traditionally used for cold and malarial fevers [9], as an anti-pyretic tonic and for the treatment of skin allergies and to cure eczema and sores [10] It is also used in traditional medicine for treating diabetes.[11] The present study was designed to investigate the different pharmacological potentials of *Artemisia roxburghiana*, including anti-inflammatory, anti-diabetic and anti-oxidant.

METHODOLOGY

Plant Collection and Identification

Artemisia roxburghiana was collected from Swat during the month of June to September 2015 and was properly identified.

Extraction

The whole plant was washed under running tap water for several times. After washing the plant material it was air dried under standard conditions for the period of three weeks to avoid any contamination. After drying the plant material was crushed into fine powder by using grinding mill. The whole powdered plant was stored in air tight bottles at room temperature and then the plant was subjected to solvent extraction. For extraction of active compounds 20 g, 50 g and 100 g of the whole plant powder was macerated with 150, 300 and 500 ml solvents of varying polarities, ranging from non-polar to polar. Following seven solvents were used: N-hexane (NH), Methanol (M), Ethanol (E), Pet-Ether (PE), Chloroform (Ch), Acetone (Ace), and distilled water (Dw). The whole plant material was weight and then shook in shaker for the period of 72 hours at 37°C and 150 rpm. At the end of extraction process the plant was filtered first through muslin cloth (coarse filtration) and then with filter paper Whattman no.1 (fine filtration). Filtrate was concentrated under reduced pressure (40 ^{o}C) using rotary evaporator and dried by using water bath. The crude plant material was stored in tight jars at room temperature for further processing.

Qualitative Phytochemical Analysis

Phytochemical tests were carried out using the methods reported by Sofowara (1993),[12] Trease and Evans (1989),[13] and Harborne (1973).[14]

Total Phenolic Content

The estimation of total Phenoli contents was carried out in 96 well plate by using Folin Ciocalteu method.[15] Gallic acid was used as standard. The absorbance was calculated by the given formula.

$$\mu g\ GA/mg\ of extract = \frac{|of|sample}{\frac{|of|standard}{400}} \times 1000 \times conc.\ of standard$$

Total Flavonoid Content

Total Flavonoidontents were estimated by Aluminium chloride method [16] the compound Quecertin was used as standard against it. The absorbance was calculated by the formula as given below.

$$\mu g\ QE/mg\ of extract = \frac{|of|sample}{\frac{|of|standard}{400}} \times 1000 \times conc.\ of standard$$

Antioxidant Assays

The antioxidant study of Artemisia roxburghiana plant extracts N-hexane (NH), methane (M), ethane (E), pet-ether (PE), Chloroform, Acetone and distilled water (4mg/mL) was carried out by using the following procedures.

DPPH (2, 2' – Diphenyl Picrylhydrazyl) free radical scavenging assay

The 2, 2'– Diphenyl Picrylhydrazyl (DPPH) radical scavenging assay was carried out by using the standard procedure with slight modifications.[17] The samples showing percent scavenging of DPPH more than 50 were further analyzed at lower concentration for calculation of IC50 values. Percentage scavenging of the DPPH free radical was measured using the following equation

$$DPPH\ radicalscavenging = 1 - \frac{absorbance of sample}{absorbance of control} \times 100$$

Total Antioxidant Capacity (TAC) Assay

TAC assay was performed by using Phosphomolybdenum method. Dimethyl sulfoxide (DMSO) was used as negative and ascorbic acid final concentration 50 µg/mL was used as standard. The graph was plotted extracts vs. µg/mL concentration of Ascorbic acid.

Total Reducing Power (TRP) Assay

A mixture of plant extract, 200 µL Phosphate buffer (0.2 M, pH 6.6) and 250 µL Potassium Ferricyanide (1%) was incubated at 50oC for 20 minutes. After adding 200 µL Trichloroacetic acid it was then centrifuged at 3000 rpm for 10 min. After that the supernatant was mixed with 50 µL FeCl3 (0.1%). The absorbance was taken at 630 nm in UV-Visible spectrophotometer. Ascorbic acid (100 µg/mL as final concentration) was used as positive standard and dimethyl sulfoxide (DMSO) as negative.

Anti-inflammatory Activity

Formalin induced right hand paw edema [18] method was used to investigate anti-inflammatory activity of Artemisia roxburghiana extracts on male Albino mice of body weight 35–40 g. The animals were divided into 10 groups of 3 mice each. The inflammation was induced by giving single injection of 100µl (0.1 mL) of 2.5% freshly prepared Formalin solution injected into sub plantar tissue of right hand paw for the purpose of inflammation. Dexamethasone as standard drug at concentration of 3mg/kg body weight was used. The extracts 2.5 mL/day were administered orally for the period of one week. The paw thickness of each mice was recorded from day 0 to 6. Inhibition of edema in percentage was calculated by following equation:

$$Inhibitionofedema = [(C0 - Ct)/C\ 0]x100$$

C0= represent edema of control group measured as average
Ct= edema of test group measured as average

Anti-diabetic Activity

Alloxan-induced Anti-diabetic study

The method reported by Aruna and coworkers (1999)[19] was used to investigate in-vivo antidiabetic potential of Artemisia roxburghiana extracts on Wister Albino rats. The adult male Wistar albino rats of body weight ~250 g were obtained from animal house NIH, Islamabad. Diabetes was induced by a single injection of Alloxan monohydrate 150 mg/kg and the fasting blood glucose level of 220–500 mg/dL were considered as diabetic. Prepared extracts were administered orally at the concentration of 5mL/day and blood glucose levels were measured by Glucometer on 11th, 22nd, 30th day of treatment period. A graph was plotted blood sugar vs time.

α – Amylase Inhibition Assay

α–amylase inhibition assay was performed in 96 well plate. 15 µL of Potassium phosphate buffer (pH 6.8) and 25 µL of α amylase enzyme was added in all wells. After that 10 µL of sample was added along with 40 µL of Starch solution. % inhibition of α amylase was calculated by the following formula.

$$inhibition = \frac{(ODx - ODy)}{(ODz - ODy)} \times 100$$

ODx= absorbance of sample
ODy= absorbance of negative control
ODz= absorbance of blank

RESULTS

Table–1 depicts the qualitative phytochemical screening of Artemisia roxburghiana (A. rox) in seven different extracts. Mean±SD quantitative total flavonoid and phenolic content (TFC & TPC) phytochemical results of Artemisia roxburghiana plant extracts is given in Table–2 showing significant difference between the extracts and standard used. RSA (DPPH radical scavenging activity), total antioxidant capacity (TAC), and total reducing power (TRP) of Artemisia roxburghiana extracts at final Conc. 400 µg/mL is depicted in Table–3. $p \leq 0.05$ are considered as significant.

DISCUSSION

The present study was designed to investigate the A. roxburghiana active substance, to investigate its photochemistry and its pharmacological properties as anti-inflammatory, anti-oxidant and anti-diabetic. The literature disclose that flavonoids are large groups of phenols with multiple pharmacological properties as they are strong antioxidant by scavenging free radicals as it can inhibit the enzymes that are responsible for the production of Reactive oxygen species. It is also helpful in the treatment of diabetes. Flavonoids are antitumor by the process of kinase enzyme inhibition and inhibit the process of apoptosis.[20] Terpenoids have anti-inflammatory, anti-allergic and antimicrobial activity. Saponins are involved in blood coagulation process. The glycosides are class of plant components which are present in all medicinal plants with large number of therapeutic effects. The Alkaloids are class of chemical compound that have role in treatment of nervous disorder as its decease appetite. Steroids have property of reducing blood cholesterol and immunogenic properties.[21] Many plant compounds which have pharmacological properties are extracted in organic solvents, like ethanol and methanol.[22] The qualitative phytochemical analysis of A. roxburghiana for the presence and absence of bioactive compounds was carried out by using the standard procedure. The results showed the presence of Alkaloids, Phenolic, Flavonoids, Saponins, Terpenoids, Reducing Sugars, Glycosides, Steroids and carbohydrates. The Flavonoids and steroids were presents in all the extracts. The Quantitative antioxidant assays were carried out for the estimation of Total Phenolic Contents (TPC), Total Flavonoid Contents (TFC), DPPH radical scavenging property, Total antioxidant capacity (TAC) and Total reducing power (TRP) of A. roxburghiana. The results showed that the quantity of TPC was high in Methanol (142.90±0.007) while TFC were high in Ethanol (306.00±0.01). Rashid et al., (2010) reported TPC and TFC close to the results of present study.[23] The antioxidants from the natural source are of great interest as they protect the human body from harmful effects of free radicals. To investigate different antioxidants in medicinal plants various procedures are used. DPPH 1, 1-diphenyl-2-picrylhydrazyl, a free radical used for measurement of free radical scavenging activity of different extracts at particular wavelength. When protons donated by the antioxidants to the free radical presents in the extracts the absorption decreases.[24] The antioxidant assays showed that the plant distilled water extract has high radical scavenging properties (86.83±0.007), TAC was high in ethane (8.38±0.33) and TRP in methane (9.99±0.28). The results of present antioxidant study are supported with the reported work of Erel in 2010 [25]. He reported

No.	Name of Tests	N-hexane	Methane	Ethane	Pet-Ether	Chloroform	Acetone	Distilled Water
1	Tannins	-	+	+	-	+	-	+
2	Alkaloids	+	-	-	+	+	-	-
3	Cardioglycosides	+	-	-	+	+	+	-
4	Flavonoids	+	+	+	+	+	+	+
5	Saponins	-	+	+	-	+	-	+
6	Phenolics	-	+	+	-	+	+	-
7	Reducing suger	-	+	+	-	+	-	+
8	Volatile oils	-	-	-	-	+	+	-
9	Carbohydrates	+	-	-	+	+	-	+
10	Steroids	+	+	+	+	+	+	+
11	Anthraquinons	-	-	-	-	-	-	-
12	Terpenoids	+	-	-	+	+	+	-
13	Amino acids	-	-	-	-	-	-	-

Table 1 Results for Qualitative Phytochemical Screening of Artemisia roxburghiana (A. rox) in Seven Extracts

S No	Extracts	Total Flavonoid Content (TFC) µg QE/mg of extract	Total Phenolic Content (TPC) µg GAE/mg of extract
1	N-Hexane	80.36±0.05*	42.81±0.01*
2	Chloroform	148.71±0.003**	135.87±0.006**
3	Pet-Ether	107.66±0.03	109.98±0.13**
4	Distilled Water	21.95±0.003**	88.06±0.006**
5	Methane	236.35±0.01**	142.90±0.007**
6	Ethane	306.00±0.01**	99.04±0.004**
7	Acetone	146.83±0.01**	123.52±0.007**

*Values are expressed as Mean ± SD (*p < 0.05, **p < 0.01)*

Table 2 Mean+SD Quantitative (TFC & TPC) Phytochemical Results of A. rox Plant Extracts.

S. No	Extracts	DPPH reducing scavenging % activity	IC 50%	TAC (µgAA/mg of AA)	TRP (µgAA/mg)
1.	N-Hexane	12.51±0.02	38.59	5.45±0.23	6.51±0.04#
2.	Chloroform	59.20±0.02*	49.78	8.29±0.28*	9.81±0.14*
3.	Pet-Ether	21.50±0.04*	19.25	8.21±0.45	9.34±0.28#
4.	Distilled Water	86.83±0.007*	6.98	5.52±0.20*	8.86±0.10*
5.	Methane	85.15±0.02*	18.60	8.09±0.33*	9.99±0.28#
6.	Ethane	82.77±0.007*	19.54	8.38±0.33*	9.98 ±0.28*
7.	Acetone	69.91±0.01*	48.97	8.37±0.32*	9.86±0.18*

Table 3: Reducing Scavenging Activity (DPPH radical scavenging activity), TAC (total antioxidant capacity), TRP (total reducing power) of Artemisia roxburghiana extracts at final Concentration 400 µg/mL.

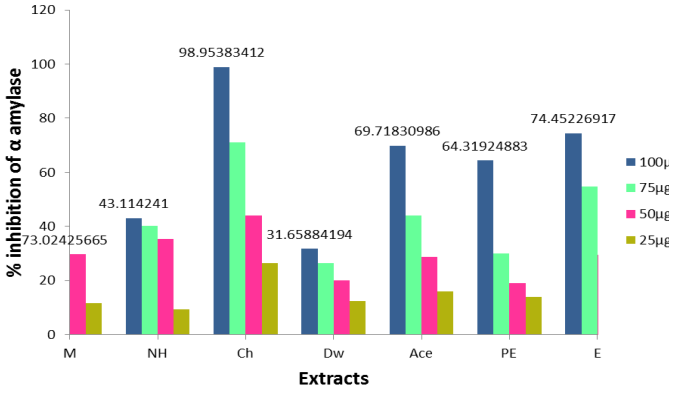

Figure 1 Roxburghiana % Inhibition of α-amylase Antidiabetic activity

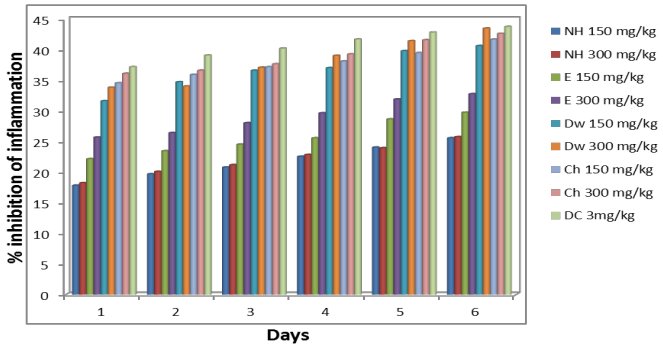

Figure 2 A. roxburghiana four extracts N-hexane (NH), Ethanol (E), Distilled

Groups	Blood Glucose			
	Day 0	Day 11	Day 22	Day 30
Normal Control	72±2.64	71.52±0.24	70.99±4.0	69.66±4.61
Diabetic Control	459.33±29.83	434±10.00*	408±13.22*	384±23.06*
Giblenclamide	403.33±16.04	207±10.58*	114±7*	74.33±2.08
DW 200 mg/kg b.w.	424.66 ±23.71	373.23±13.61*	244.13±47.38*	125±9.29
DW 400 mg/kg b.w.	408±17.43	327.19±18.17*	191.03±14.15*	105±5.29*
E 200 mg/kg b.w.	446.33±33.85	332.06±21.12*	181±13.07*	92.33±6.65*
E 400 mg/kg b.w.	411±9.64	301.14±11.37*	164±24.5*	87.66±16.92*

*Values are expressed as Mean + SD (*p < 0.05 are considered as significant)*

Table 4 Effects of Different Treatments on Blood Glucose level of Rats. The Data is given as mean ± SD of Triplicates

the antioxidant and DPPH scavenging properties of Artemisia. Diabetes is a worldwide problem which characterized by insulin deficiency. A. roxburghiana is traditionally used for the treatment of diabetes.[11]

Alloxan exerts its action when it's delivering by parenteral way and causes destruction of pancreas Beta cells and results in an increase of blood glucose level and level of insulin decreases. The increase of blood glucose level is due to the process of gluconeogenesis in the absence of insulin.[26] The Ethanolic extract show significant antidiabetic activity as compared to Distilled water extract. The similar results are reported for Artemisia species.[27] There is no reported activity for A. roxburghiana therefore the results discussed with other species of the genera. The α–amylase inhibitory activity of A. roxburghiana was investigated. α amylases are enzymes of pancreatic cells that causes digestion of carbohydrates and results an increase in blood glucose level. These are most active form of Alpha enzymes found in human. In medicinal plants some flavonoids are present which can interfere with these enzymes and inhibit its activity.[28] Extracts show significant inhibitory activity for α–amylase. From all the extracts distilled water show excellent inhibitory activity (98.95%). The extracts have shown inhibitory activity (59.2±0.11%) at the concentration of 100 µg/mL. Response of tissues towards deleterious stimuli and pathogens and dead cells is called inflammation.[29] Formalin is toxic and can cause swelling, redness and pain. The Chloroform show significant % inhibition of inflammation close to standard drug. Jaleel and group in 2015 studied anti-inflammatory activity of Artemisia in mice.[30] The ethanolic extract of Artemisia was used at 100, 200 and 400 mg/kg dose. The extract showed percentage inhibition 62.5%, 35% and 46.8%. Similar trials were carried out [31,32] and encouraging results were obtained and are consistent with the present study.

CONCLUSION

Results showed that RSA (DPPH radical scavenging activity), total antioxidant capacity (TAC), and total reducing power (TRP) of Artemisia roxburghiana extracts at final concentration of 400 µg/mL is considerably significant. It is further concluded that this plant is a source of valuable natural products for pharmaceutical industries and can be used for identification and isolation of novel compounds which leads toward drug discovery for treatment of more serious and complicated diseases.

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