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Research Article

ANTIOXIDANT, ANTIDIABETIC AND ANTIBACTERIAL ACTIVITIES OF *RICINUS COMMUNIS* ROOT EXTRACTS

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ABSTRACT

Present study investigated antioxidant, antidiabetic and antibacterial effectiveness of *Ricinus communis* root. Total phenols content (TPC), total flavonoid contents (TFC) and antioxidant activity were determined by folin-ciocalteu reagent method, aluminium chloride colorimetric technique and 2, 2-diphenyl l- picrylhydrazyl (DPPH) scavenging assay respectively. Antiglycation, alpha amylase inhibition, and antibacterial analyses were done by prescribed procedures. Methanol fraction had significant ($P<0.05$) TPC (90± 0.19 mg/g GAE) and TFC (32 ± 0.85 µg/mL CE) along with significant ($P<0.05$) antioxidant activities (41-42%). Extracts presented optimal antidiabetic and antimicrobial efficacies validating therapeutic properties of *R.communis* root.

Keywords: Antiglycation; Alpha amylase; *E.coli*; *Staphylococcus aureus*

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1. INTRODUCTION

Numerous plant products are used extensively in traditional medicinal systems. Diverse pharmacological efficacies of plant derived secondary metabolites has greatly excelled the focus of scientists redefining new research domains [1].

Ricinus communis (Euphorbiaceae) commonly called castor is a vital constituent in Ayurvedic medicines for different diseases [2]. The presence of secondary metabolites in *Ricinus communis* attributes diverse medicinal properties against a variety of ailments [3]. Polyphenols inhibit mutagenesis and carcinogenesis. Phytoconstituents are responsible for antioxidant activity [4]. Flavonoids can function as reducing and antimicrobial agents, destroyers of reactive oxygen species, harmful oxidation products, and participates in chelation processes [5]. Methanolic extract of *Ricinus communis* leaves considerably prevented hepatitis, enhanced the liver restoration and hence, it could be used in drug development [6]. The lectin rich *R. communis* seed protein has strong antibacterial and anticancer activities [7].

In uncontrolled diabetes mellitus, hyperglycemia leads to glycation process and secondary complications. The use of amylase inhibitors can ameliorates hyperglycemia. Synthetic glycation and alpha amylase inhibitors either have side effects or their prolong usage becomes ineffective due to drug resistance [8]. Administration of medicinal plants during *in vivo* studies reflected glucose and lipid lowering potentials. Although the actual antidiabetic mechanism of most natural products is still unknown, nonetheless, administration of plant extracts imitate dominant antidiabetic outcomes [9]. *Ricinus communis* has recently been reported to possess analgesic, antihistamine, antioxidant and anti-inflammatory activities [7].

Ricinus communis possess strong antimicrobial activity against *Aspergillus niger, Bacillus subtilis, A. solani, Salmonella typhi, Staphylococcus aureus, F.oxysporum, Enterobacter, Pseudomonas aeruginosa* and *Escherichia coli* [10-12]. Although *Ricinus communis* has been extensively studied for various biological activities, mostly aerial parts of the plant were analyzed. Limited data is available regarding *Ricinus communis* roots. In addition, data on comparative efficacies of plant extracts in organic solvents is limited. Hence, present study was conducted to investigate antioxidant, antidiabetic and antibacterial activities of *Ricinus communis* roots in various organic fractions.

2. EXPERIMENTAL

2.1. Plant Materials and Sample Preparation

Ricinus communis (Castor) collected randomly from Faisalabad were identified and authenticated at the Department of Botany, University of Agriculture, Faisalabad, Pakistan. Shade-dried, powdered root sample was soaked in methanol. Methanol was evaporated and semi-solid viscous sample was dried on water bath. Methanol extraction was done three times four days apart. Fractionation into five solvents and water was performed [13]. Reagents for antioxidant profile, antidiabetic and antibacterial activities were obtained from Sigma Aldrich (Germany). Rifampicin was purchased from Applichem.

2.2. Phytoconstituents

2.2.1. Total Phenolic Contents (TPC)

TPC were determined with folin-ciocalteu reagent [14]. Briefly, sample and reagent were incubated at room temperature for 5 minutes. Then Na_2CO_3 was added and the resulting mixture was boiled for 20 minutes. Absorbance was measured at 750 nm and results were expressed in terms of gallic acid equivalent (mg gallic acid/g dry weight).

2.2.2. Total Flavonoid Contents (TFC)

Aluminium chloride colorimetric technique was used for TFC estimation [15]. Sample and $NaNO_2$ were incubated at room temperature for 10 minutes. Then $AlCl_3$ and NaOH were added and again incubated at room temperature for 20 minutes. Absorbance was measured at 510 nm and TFC were expressed as µg/mL catechin equivalents.

2.2.3. DPPH radical scavenging assay

The antioxidant activity was assessed in terms of 2, 2-diphenyl l- picrylhydrazyl (DPPH) scavenging abilities [16]. Test sample and DPPH solution were incubated at room temperature for 30 minutes. Absorbance at 517 nm was used to calculate radical scavenging activity as: IC_{50} (inhibitory concentration; %) = (Absorbance blank – Absorbance sample / Absorbance blank) x 100.

2.3. Antidiabetic activity

2.3.1. Antiglycation assay

Antiglycation test was performed as described by Matsuda et al. [17]. Briefly, glucose and bovine serum albumin were incubated at 37 ^{o}C for 11 days with or without the test sample. The absorbance was measured at 440 nm. The reaction mixture without D-glucose was used as a blank solution. Percent glycation inhibition was calculated as: 100 - [(Optical Density (test) / Optical Density (control) × 100]

2.3.2. Alpha amylase inhibition assay

The alpha amylase inhibition assay was carried out using porcine pancreatic α-amylase [18]. Test sample and enzyme solution were incubated at 25°C for 10 minutes. Starch solution was added and mixture was then incubated at 25°C for 120 minutes. After addition of 3,5dinitrosalicylic acid (DNS) reagent, again incubated in boiling water bath for 5 minutes. Absorbance was measured at 540 nm and percent enzyme inhibition was calculated as: $Absorbance_{Control}$ - $Absorbance_{Sample}/Absorbance_{Control}$ x 100

2.4. Antibacterial activity

Antibacterial activity was assessed through well diffusion method [19] against *Escherichia coli, Staphylococcus aureus* and *Pasteurella multocida*. Strains were procured from Industrial Biotechnology Laboratories, Department of Biochemistry, University of Agriculture, Faisalabad. Muller Hinton agar media was inoculated with microbial strains. Plant extract fractions were applied and incubated at 37°C. Rifampicin was used as positive control. The diameters of the inhibition zones were measured. Data was expressed as mean ± standard deviation (SD), percentage or n. Statistical analysis was done by SPSS (version 14) with level of significance set at $P \leq 0.05$.

3. RESULTS

3.1. Phytoconstituents and antioxidant activity

TPC were 23± 0.26 - 90± 0.19 (mg/g gallic acid equivalents) among methanol, ethanol, *n*-butanol, ethyl acetate, *n*-hexane, chloroform and aqueous fractions. Methanol fraction had significant ($P<0.05$) TPC (90 ± 0.19 mg/g gallic acid equivalents). TPC in other extract and fractions in ascending order were as *n*-

butanol<ethyl acetate< *n*-hexane<ethanol<aqueous as presented in Table.1. Methanol was most potent solvent (P<0.05) in extracting maximum TFC (32 ± 0.85 µg/mL catechin equivalents). In other samples, TFC in ascending order was as *n*-butanol>aqueous>ethanol>ethyl acetate> chloroform> *n*-hexane (Table.1). Significant (P<0.05) antioxidant activity was shown by methanol and aqueous extracts (41-42%). While other samples showed activity in descending order as n-butanol>ethanol>chloroform>n-hexane>ethyl acetate (table 1).

3.2. Antidiabetic activity

Maximum (52%) glycation hindrance (P<0.05) was shown by methanol extract followed by aqueous extract (P<0.05). Almost analogous glycation restriction potential (33-37%) was revealed by *n*-butanol, ethyl acetate and ethanol fractions. While, synthetic inhibitors reduced the process by 19-47%. The pattern in descending order was as *n*-butanol>methanol>aqueous>ethanol>chloroform> *n*-hexane fractions (Table.1). Aqueous and methanolic extracts were most reactive inhibitors displaying 33 and 39.5% (P<0.05) inhibitions of alpha amylase activities respectively. For other solvent fractions, percent decrease in activity in descending order was as following: *n*-butanol> ethanol≥ chloroform> *n*-hexane>ethyl acetate (Table.1).

Table1. Phytoconstituents, antioxidant and antidiabetic activity of *Ricinus communis* root extracts

Extract/ Fractions/ Synthetic compounds	**Phytoconstituents**		**Antioxidant activity**	**Antidiabetic activity (%)**	
	TPC	**TFC**	**(IC_{50}) %age**	**Glycation Inhibition**	**Alpha amylase Inhibition**
Methanol	90 ± 0.19	32 ± 0.85	41	52	39.5
Ethanol	42 ± 0.23	23 ± 0.89	22	33	20
Ethyl acetate	30 ± 0.31	22 ± 1.17	10	32	13
n-butanol	23 ± 0.26	26 ± 1.23	23	37	22
n-hexane	35 ± 0.16	14 ± 0.93	12	20	15

Aqueous	78 ± 0.36	25 ± 0.79	42	44	33
Chloroform	14 ± 0.32	19 ± 0.99	18	27	20
BHT	-	-	66.23	-	-
Niacin	-	-	-	47	-
Metformin	-	-	-	32	-
PABA	-	-	-	19	-
Acarbose	-	-	-	-	47

Data expressed as percentage (%) or mean ± SD (standard deviation). TPC: total phenolic contents expressed mg gallic acid equivalent /g dry weight; TFC: total flavonoid contents expressed as µg/mL catechin equivalents; DPPH: 2, 2-diphenyl l-l-l-picrylhydrazyl, BHT: Butylated hydroxy toluene (positive control), PABA: para amino benzoic acid, Niacin, Metformin and PABA: synthetic glycation inhibitors, Acarbose: synthetic alpha amylase inhibitor.

3.3. Antibacterial activity

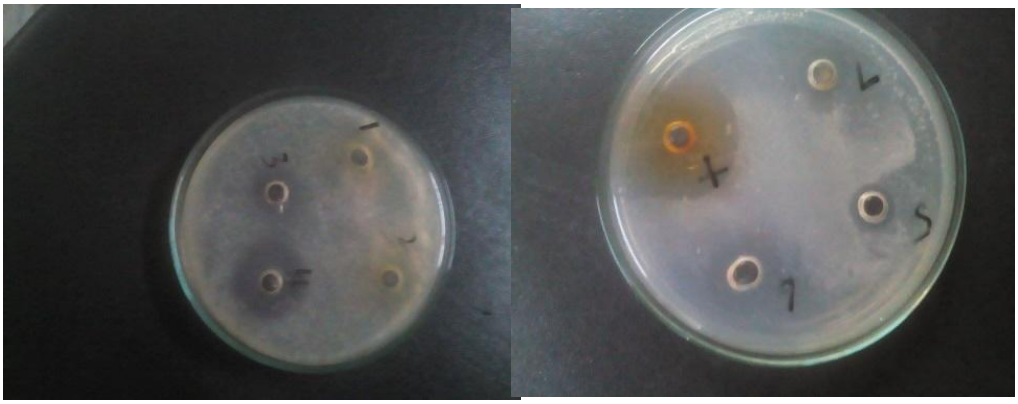
Results are depicted in table. 2 and fig. 1-3. For *E.coli*, all extracts inhibited growth except methanol and ethanol samples that were bacteriostatic. Maximum growth inhibition was shown by chloroform fraction (fig. 1).

In case of *S. aureus*, methanol and ethanol extracts were bacteriostatic while all other samples inhibited bacterial growth with inhibition range of 20-33 mm (fig. 2). Sample show bacteriostatic activity when minimum bacterial concentration (MBC)/minimum inhibitory concentration (MIC) ratio is > 4. *n*-hexane fraction was the most potent antimicrobial ($P<0.05$) sample against *Staphylococcus aureus*. Most of the samples tested were bacteriostatic towards *Pasteurella multocida* (fig.3). Methanol, ethanol and chloroform fractions revealed minimal growth inhibitory effects (4-12 mm). Overall, impressive bacterial growth restriction for all tested strains was exhibited by chloroform fraction ($P<0.05$). Results also showed that *P.multocida* was least susceptible bacterial strain against tested fractions. However, the vulnerability portrayed by the tested bacteria towards root extracts needs to be further explored for medicinal uses.

Table 2. Antimicrobial profile

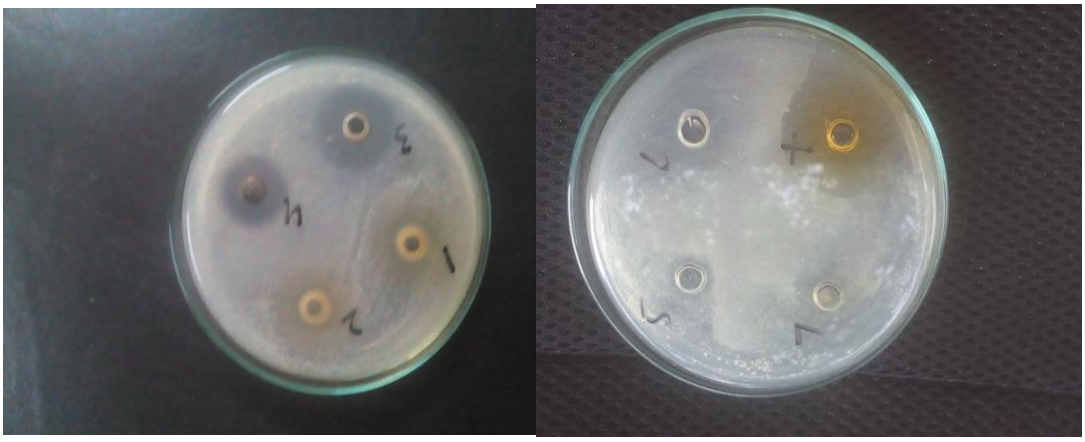
Sr. No.	**Extract**	**Zone of inhibition (mm)**		
		E. coli	***S.aureus***	***P.multocida***
+	Rifampicin (positive control)	27	43	38
1	Methanol	Bacteriostatic	Bacteriostatic	4
2	Ethanol	Bacteriostatic	Bacteriostatic	6
3	*n*-butanol	15	28	Bacteriostatic
4	Aqueous	17	23	Bacteriostatic
5	Ethyl acetate	15	29	Bacteriostatic
6	*n*-hexane	12	33	Bacteriostatic
7	Chloroform	19	20	12

Data represented as millimeter (mm) inhibition zones or as bacteriostatic effects. Bacteriostatic: means that the extracts prevented the growth of bacteria (i.e. it kept them in the stationary phase of growth), Bactericidal: means that extracts killed bacteria.



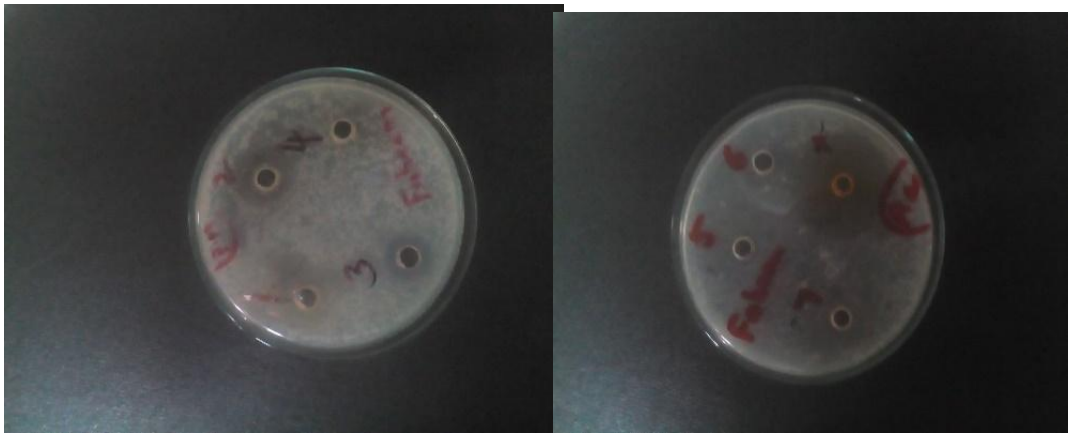
(a) **(b)**

Fig. 1(a-b). Antimicrobial profile – *E. coli*



(a) **(b)**

Fig. 2(a-b). Antimicrobial profile - *Staphylococcus aureus*



(a) **(b)**

Fig. 3 (a-b). Antimicrobial profile - *Pasteurella multocida*

1: methanol extract, 2: ethanol extract, 3: *n*-butanol, extract, 4: aqueous extract, 5: ethyl acetate extract, 6: *n*-hexane extract, 7: chloroform extract, +: positive control (rifampicin).

4. DISCUSSION

Measurement of phytoconstituents reflects medicinal potential of plants. Current TPC inferences are in accordance with the previously reported TPC (29.6 mg/g gallic acid equivalents) by Yadav and Agarwala [20]. Similarly, TFC results were similar to the findings of Rao *et al.* (2013), as they determined 17-28 µg/mL catechin equivalent TFC in *Ricinus communis* leaf extract. Ilavarasan et al. [21] observed significant ($P<0.001$) antioxidant activity of methanolic

extracts of *Ricinus communis* roots in rat models. It is suggested that various extracts and fractions of *Ricinus communis* roots contain antioxidant components and possess antioxidant activities, with methanol being the most powerful solvent in extracting optimum chemical constituents.

Antidiabetic potentials of *R. communis* roots presented in current research can be justified by the fact that flavonoids and phenols inhibit glycation process as well as enzyme related to carbohydrate metabolism [17]. Limited data is available regarding the antiglycation and alpha amylase inhibitory potentials of root extracts of *R. communis*. Shokeen et al. [22] investigated the antidiabetic activity of 50% ethanolic extract of roots of *R. communis* along with its bioassay-guided purification. Out of several different fractions tested, only one fraction showed significant antihyperglycemic activity. Thus, *R.communis* roots seem to have a promising value for the development of a potent phytomedicine for diabetes.

Antibacterial results were in accordance with the previous studies. *Ricinus communis* possess strong antimicrobial activity [10-12]. Verma et al. [23] screened root extracts of *Ricinus communis* against pathogenic microorganisms such as *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhimurium, Proteus vulgaris, Bacillus subtilis, Candida albicans* and *Aspergillus niger* using well diffusion method. The findings suggested that the roots extracts were effective antimicrobial agent. Sharma et al. [24] reported antimicrobial activity by leaf extracts of *Ricinus communis* against *S. aureus, K. pneumonia and P. aeruginosa.*

5. CONCLUSIONS

In conclusion, the tested solvent fractions of *R.communis* roots possessed variable antioxidant, antidiabetic and antibacterial activities with methanol being the most potent solvent in extracting maximum phyto-constituents. Although present study establishes the therapeutic rationale of *R.communis* root utilization as herbal formulations, bioassay guided isolation and characterization of active components and further *in vivo* studies can disclose compounds with better therapeutic value.

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