WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 11, 168-176. Research Article ISSN 2277– 7105

PHYTOCHEMICAL AND BIOLOGICAL EVALUATION OF *ALBIZIA RICHARDIANA* BENTH, FABACEAE FAMILY

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Article Received on
24 Aug 2015,

Revised on 17 Sep 2015,
Accepted on 11 Oct 2015

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ABSTRACT

This dissertation aimed to investigate and evaluate the possible phytochemical and biological profiles like, phytochemical screening, antioxidant, antimicrobial, cytotoxic and hypoglycemic activities of the medicinal plant *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae). Phytochemical screening test of the methanolic plant extract of *Albizia richardiana* suggests the presence of bioactive substances like- carbohydrates, saponin, glycosides, glucosides, alkaloids in the bark extract of plant. Antioxidant activity was screened using DPPH assay and ethyl acetate soluble partitionate of bark revealed the significant free radical scavenging activity with IC50 values of 17.8 µg/ml as compared to standard, butylated hydroxyl toluene (IC50 11.62 µg/ml). In the brine shrimp lethality bioassay, the chloroform soluble extractive from bark showed cytotoxicity (LC50 = 22.03 µg/ml) as compared to vincristine sulfate (LC50 = 0.52 µg/ml). Significant hypoglycemic activity was observed after 60 and 120 minutes of administration. No significant antimicrobial was observed from this plant part. Chemical investigation of the bark extract of *Albizia richardiana*, for the first time from this plant. This is the also the first report of the bioactivities of this plant.

KEYWORDS: Antioxidant activity, Phenolics, Flavonoids, Terpenoids, Saponins, Cytotoxicity.

INTRODUCTION

Albizia richardiana (Fam. Fabaceae, subfam. Mimosoideae) grows naturally in Asia, Africa, Madagascar, America and Australia.[1,2] The Mimosoideae include a subfamily of the flowering plant family Fabaceae (Leguminosae) categorized by flowers with small petals and numerous noticeable stamens.[3] They are commonly called silk plants, silk trees and sometimes the plants may be entitled as albizzias. They are typically trifling trees or shrubs with a short lifespan. The flowers, bark, fruits, roots, and stems of Albizia richardiana are all used for medicine.[4] Their major function includes depression for tranquilization, dispersing wind to improve eyesight, increase appetite, promoting blood circulation, chest tightness, wind-fire eye problems, blurred vision, back pain, and injuries from falls. [5] Phytochemical study of different Albizia species revealed the company of different classes of secondary metabolites, such as saponins, terpenes, alkaloids and flavonoids. The aglycon part perhaps a steroidal or triterpenoidal nucleus that is attached to one or more sugar, most often composed of D-glucose, L-arabinose, D-galactose, D-glucuronic acid, L-rhamnose, D-xylose or D-fucose. Saponins have been used widely in pharmaceutical industries for their pharmacological importance.[6-8] The work elucidated in this dissertation is an attempt to evaluate the possible biological profiles of the medicinal plant *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae). The genus of the plant is Albizia. The other plants of this genus have various significant medicinal properties for instance they are recognized to treat asthma, arthritis, antiseptic, burns, antidysentric, allergic rhinitis etc. But no significant amount of work has been done on the plant *Albizia richardiana* of this genus. Therefore, the present study was designed to investigate the phytochemical screening, antioxidant, antimicrobial, cytotoxic and hypoglycemic activities of the crude methanolic extract of bark of *Albizia richardiana* and report the results of our investigation, so that it can be given the recognition of medicinal plant.

MATERIALS AND METHODS

Drugs and chemicals

Methanolic acid, Glibenclamide, 10 mg tablet, Tween-80, diclofenac sodium, normal saline solution, DMSO, freshly prepared 10% of alcoholic solution of alpha naphthol, conc. sulphuric acid, Fehling's solutions A and B, dilute hydrochloric acid, sodium hydroxide solution, 5% ferric chloride solution, acetic anhydride, 3% copper sulphate solution, potassio-mercuric iodide solution, 1% solution of picric acid, saturated solution of I_2 in KI, bismuth

potassium iodide solution, 10% Tannic acid solution**,** vincristine sulphate were used in this investigation.

Plant materials

Bark of *Albizia richardiana* was collected from the Chittagong hill area, Bangladesh, as plant sample. The sample was then identified by an expert taxonomist at S.K.H.D.U (Acc. No.10778). Then it was cut into small pieces for better drying as we know evaporation increases with the incensement of the surface area. After that it was sun dried for several days which is then followed by oven drying. The dried bark was then crushed into coarse powder by a high capacity grinding machine, which was well cleaned before grinding to avoid cross contamination. Two amber containers (2.5) liters were used for soaking 500 g powdered plant materials (bark) in 1.2 liter of methanol as solvent. These two containers together with its contents were sealed by cotton plug and aluminum foil. Then these two containers were reserved like this in a dry place for about a period of two weeks with occasional shaking and stirring. After two weeks whole mixture was filtered, at first through cotton and after that through Whatman No. 1 filter paper. From this filtration, about 525ml filtrate was obtained. The filtrate volume was then reduced using a Buchii Rotavapour at low temperature and pressure, after which, the final volume of the filtrate was 100 ml. Then the filtrate was kept in a beaker and sealed with aluminum foil with several pores on it. After that the filtrate was kept for drying to become crude dry extract. The weight of final extract was 12 g.

Animals

For this experiment, Swiss-albino mice of either sex, aged 4-5 weeks, were used which were obtained from the Animal Resource Branch of the International Centre for Diarrheal Diseases and Research, Bangladesh (ICDDR'B). Some necessary works had to be done, for instance- mice were housed in standard polypropylene cages, the room temperature was 24±2°C, relative humidity 60-70%, in a 12hr light-dark cycle and their food was rodent food and their water was formulated by ICDDR'B. These animals were kept in the environment where the experiment took place before the test for at least 3-4 days, as they are very sensitive to environment changes.

Investigation of chemical compounds by phytochemical screening

The methanolic bark extract of *Albizia richardiana* was subjected to analysis chemical screening for the identification of the presence of bioactive substances like, carbohydrate, tannin, glycosides, alkaloids, and saponins etc. by using standard procedures.

Antioxidant activity

Antioxidant activity of the methanolic bark extract of *Albizia richardiana* was evaluated by 1, 1-dipheny-l-2-picryldrazyl and estimated by testified methods.[9] Butylated hydroxyl toluene was used as standards and DPPH solution was used as control. The absorbance was measured by UV spectrophotometer (570 nm). Inhibition of free radical was estimated by following equation:

Inhibition of free radical % = A_b-A_s/ A_b ×100 where, A_b = Absorbance of the control and A_s = Absorbance of the methanolic bark extract of *Albizia richardiana*.

IC_{50} was calculated by plotting the inhibition concentration versus standard methanolic bark extract of *Albizia richardiana* (MBE) concentration.

Antimicrobial activity

The methanolic bark extract of *Albizia richardiana* was verified for antimicrobial activities by the standardized disc diffusion method.[10] Antimicrobial activity of the methanolic bark extract of *Albizia richardiana* was tested beside some gram positive and some gram negative bacteria. Here, five gram positive bacteria namely, *B. cereus*, *B. megaterium*, *B. subtilis*, *S. lutea* and *Stap. aureus* as well as eight gram negative bacteria namely, *E. coli*, *P. aeruginosa*, *S. paratyphi*, *V. mimicus*, *S. dysenteriae* and also three fungi namely, *C. albicans*, *A. niger* and *S. cerevacae* were used. The obtained results were compared with standard ciprofloxacin.

Brine shrimp lethality bioassay

For the screening of pharmacological activities in plant extracts, Brine shrimp lethality bioassay. [11] has been suggested. Rapid, inexpensive and simplicity (eg.no aseptic techniques are required) are the advantages of this method. For statistical validation, this method easily utilizes a large number of organisms and requires no special equipment and a relatively small amount of sample. Additionally, it does not require animal serum.

Evaluation of hypoglycemic activity

For the evaluation of hypoglycemic activity, the most acceptable method is glucose tolerance test (GTT). In this medical test, glucose is given and blood samples are taken subsequently to find out how quickly the glucose is cleared from blood. The test is usually used for the purpose of determining diabetes, insulin resistance, and occasionally reactive hypoglycemia or rare disorder of carbohydrate metabolism. Many variation and development of the GTT

have been done over the years for various purposes, with different standard doses of glucose, different routes of administration, different intervals and durations of sampling with the measurement of various substances in addition to blood glucose.

Statistical analysis

In the evaluation of hypoglycemic activity, values were expressed as mean ± standard deviation and statistical comparison were done by using t-test and the $p<0.05$ value was considered as statistically significant and $p<0.001$ value as extremely significant. In brine shrimp lethality bioassay, the result value should be around of the standard value.

RESULTS AND DISCUSSION

Investigation of Chemical Compounds by Phytochemical Screening

Phytochemical screening test of the methanolic plant extract of *Albizia richardiana* suggests the presence of bioactive substances like- carbohydrates, saponin, glycosides, glucosides, alkaloids in the bark extract of plant. Results of phytochemical screening of *Albizia richardiana* is given in the following table-1.

Table-1: Result of phytochemical screening.

Test	MEB
Carbohydrates	+
Saponins	+
Glucosides	+
Glycosides	+
Flavonoids	-
Steroids	-
Tannins	-
Resins	-
Alkaloids	+
Proteins	-

MEB = Methanolic extract of bark; (+): Positive result; (−): Negative result.

Antioxidant activity

The antioxidant activity of methanolic bark extract of *Albizia richardiana* is mentioned in Table 2. The methanolic bark extract of *Albizia richardiana* showed free radical scavenging activity having significant IC_{50} of 17.8 µg/ml as compared to standard BHT (11.62 µg/ml).

Table 2. Antioxidant activity of crude extract of bark of *Albizia richardiana*.

Samples	% of Inhibition	Free radical scavenging activity IC_{50} µg/ml
BHT	66.27	11.62
MEB	39.41	17.8

BHT = tert-butyl-1-hydroxytoluene and MEB = Methanolic extract of bark.

Antimicrobial activity

The methanolic bark extract of *Albizia richardiana* showed mild to moderate antimicrobial activity against 16 microorganisms ranging from 06 to 15 mm (Table 3) as compared to ciprofloxacin (28-42 mm). The highest activity was measured against *Staphylococcus aureu* (15 mm).

Table 3. Antimicrobial activity of crude extract of bark of *Albizia richardiana*.

Test organism	Determination of zone of inhibition (mm)	
	CIP	MEB
Gram Positive bacteria		
Bacillus sereus	40	12
Bacillus megaterium	36	11
Bacillus subtilis	34	10
Sarcina lutea	39	-
Staphylococcus aureu	40	15
Gram Negative bacteria		
Escherichia coli	40	08
Pseudomonas aureus	41	06
Salmonella paratyphi	32	11
Salmonella typhi	38	09
Vibrio parahaemolyticus	42	-
Shigella boydii	37	11
Shigella dysenteriae	38	10
Vibrio mimicus	28	09
Fungi		
Candida albicans	34	06
Aspergillus niger	33	11
Sacharomyces cerevisiae	37	12

CIP= Ciprofloxacin and MEB = Methanolic extract of bark.

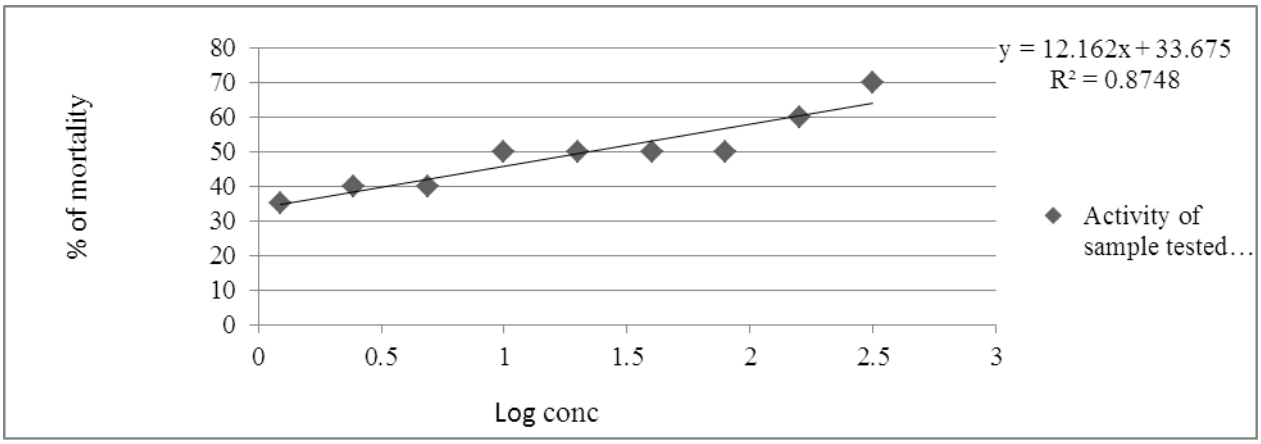
Brine Shrimp Lethality Bioassay

The methanolic extract of the plant material had LC_{50} values of 22.03µg/ml while the LC_{50} of the reference anticancer drug, vincristine sulphate was 0.52µg/ml (Table 4). With increasing concentration of the sample the rate of mortality of the nauplii had increased (Figure-1 and Figure-2).

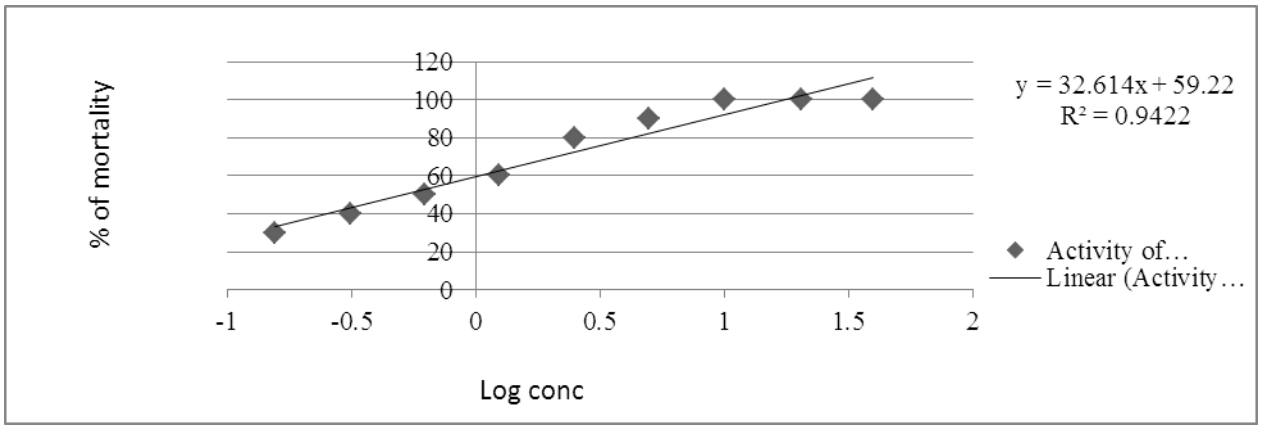
Table: 4- Antioxidant activity of crude extract of bark of *Albizia richardiana*.

Sample	LC_{50} (µg/ml)
VS	0.52
MEB	22.03

VS= Vincristine sulphate and MEB= Methanolic extract of bark.



"Fig. 1"Activity of sample tested and determination of LC_{50}.



"Fig. 2"Activity of standard and determination of LC_{50}.

Comparing the test result of the methanolic bark extract of *Albizia richardiana* with standard, it may be suggested that the plant has no significant cytotoxic activity.

Evaluation of Hypoglycemic Activity

For the evaluation of hypoglycemic activity of methanolic bark extract of ***Albizia richardiana,*** 200 and 400 mg/kg (table 5) dose of the extract were taken to observe whether they do have any effect on lowering of blood glucose level.

Table 5: Hypoglycemic activity of crude extract of bark of *Albizia richardiana.*

Sample	Plasma level of glucose (Mean)				% reduction of plasma glucose level		
	0 minute	20 minute	60 minute	120 minute	After 20 minute	After 60 minutes	After 120 minutes
CTL	5.70	10.67	7.33	5.60	-	-	-
STD	4.17	3.73	3.53	3.30	10.55^{a}	15.35^{a}	20.86^{a}
MEB 1	4.17	5.70	3.60	2.90	-36.69^{c}	13.67^{a}	30.46^{a}
MEB 2	4.30	9.13	3.90	3.50	-112.32^{c}	9.30^{b}	18.61^{b}

CLT= Control; STD= Standard; MEB 1= Methanolic extract of bark at a dose of 200 and MEB 2= Methanolic extract of bark at a dose of 400. Here, $^{a}p < 0.001$, $^{b}p < 0.05$, $^{c}p > 0.05$

considered statistically significant as compared to control group.

From the above table it is clear that, MEB1 and MEB2 do not have any significant percentage of reduction values than STD after 20 minutes of administration, but do have greater effects after 60 and 120 minutes of administration and statistically significant.

CONCLUSION

The results of the evaluation of phytochemical profile as well as biological profile show that the plant contains some of the important bioactive constituents. The methanolic extract of bark revealed the significant free radical scavenging activity with IC50 values of 17.8 µg/ml. It was also observed from the investigation that the plant extract not responded positively in brine shrimp lethality bioassay with LC_{50}value of about 22.03µg/ml. Significant hypoglycemic activity was observed, at a dose of 200 and 400 mg/kg after 60 and 120 minutes of administration. However, no statistically significant antimicrobial of methanolic bark extract of *Albizia richardiana.* Thus, the results of the present study provided a scientific support for the use of *Albizia richardiana* (Fam. Fabaceae, subfam. Mimosoideae), as a traditional medicine and deserves further extensive studies to find out bioactive constituents responsible for these activities.

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