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Comparison of Antibacterial Activities of Bark Ash with Different Solvent Extracts of Bark of the Plant *Ficus religiosa* Linn against Staphylococcus Aureus & Escherichia Coli

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Abstract

Traditional medicine in Nepal has strong cultural and religious background. It exists in different ways like ethnic and tribal group, ritual or ceremonial practices, spiritual practices, diet or self-healing practices. The medicinal plant *Ficus religiosa* (Linn) belongs to the family Moraceae, which is one of the most popular members of genus Ficus, and usually identified as Peepal. Till today use of this plant as a traditional healer is seen in majorities of the rural people for their primary health problems. As various literatures suggest about the usefulness of bark as a popular folk medication especially for cuts and wounds in rural areas of Nepal.

Here the work is aimed to compare the antibacterial activities of bark ash with respect to various solvent extracts of bark of this plant against two strains of bacteria *i.e.* Staphylococcus aureus and Escherichia coli against standard drug gentamycin.

Result suggested that antimicrobial activity of bark ash (product D & E) are more active than that of those three solvent extracts in terms of exhibiting antibacterial activities as they showed higher zone of inhibition in respect to all other extracts. The common thing found in each sample is that all the products (A to E) are significantly active against E.coli than S. aureus.

Keywords: *Ficus religiosa;* Moraceae; Peepal; Staphylococcus aureus; Escherichia coli; Gentamycin.

Introduction

The importance of medicinal plants in the national economy has been emphasized from time to time. They contain several precious bioactive compounds that form the strength of traditional medicine [1]. In Nepal, the concept of ethno-medicine has been developed since the late 19th century. Plants have made a dominant contribution for traditional medicinal systems for year after years. In ancient period, knowledge of healing powers of herbs was passed down orally, often within a family lineage. The earliest written account of herbal remedies was the Pen Ts'ao by Shen Nung of China and dates back to 2800 BC [2,3].

The medicinal plant *Ficus religiosa* (Linn) belongs to the family Moraceae, which is one of the most popular members of genus Ficus, and usually identified as Peepal. Various parts like bark, fruit, leaves and seed are widely used. It is intended for the treatment of various diseases like skin and infectious disease, cancer, inflammation and also has astringent, antiprotozol, antidiarrhoeal and antiviral activity [4]. The plant is a well-known ethno-medicinal tree, used to prepare ayurvedic dosage forms and traditional folk medicines.

Different parts of the plant are used as ethno medicine in Nepal. Bark is used as astringent, cooling, aphrodisiac, antibacterial against Staphylococcus aureus and Escherichia coli, gonorrhea, diarrhea, dysentery, heamorrhoids and gastrohelcosis, anti-inflammatory, burn. Bark decoction is used as colling, gonorehea, skin diseases, scabies, hiccup, vomiting etc. The barks of F. religiosa are used as vital ingredients in many ayurvedic formulations such as Chandanasavam, Nalpamaradi, Nyagrodhadichurna and Saribadyasavam [5].

Here the work is aimed to compare the antibacterial activities of bark ash with respect to various solvent extracts of bark of this plant against two strains of bacteria i.e. Staphylococcus aureus and Escherichia coli against standard drug gentamycin.

Materials and Methods

Plants collection

The required amount of plant material i.e. steam bark of *Ficus religiosa* (Peepal) was collected from Sunsari district Dharan 4 ground of Bhagabati School. The plant was named as *Ficus religiosa* Linn. In Post Graduate Campus, Head, Department of Botany, Tribhuvan University Biratnagar, Nepal.

Macroscopic analysis: Macroscopic evaluation was carried out visually, which provided the details concerning the plant aspect, general colour and odour appearance [6,7].

Loss on drying: About 1 g of the powdered crude drug was accurately weighed in a tared dish. Then it was dried in an oven at a temperature 100-105°C for an h. After completion of 1 h, it was cooled in a desiccator. After proper cooling, it was re-weighed. The loss on drying was calculated with reference to the amount of the dried powdered crude drug taken.

Total ash value: To determine the total ash value, about 2 g of air dried crude drug was weighed and maintained at a temperature not exceeding 45°C until it became free from carbon. After incineration, the material was cooled and weighed. The percentage of ash value was calculated with reference to the air dried powdered crude drug taken.

Thin Layer Chromatography (TLC): TLC was performed on a sheet of glass, plastic or aluminum foil, which is coated with a thin layer of adsorbent material known as stationary phase. After that the sample was applied on the plate. A solvent, solvent mixture (mobile phase) is drawn up the plate based on the mechanism of capillary action. Because of different component of mixture ascend the TLC

plate at different rates and separation is attained followed by measuring the Rf value.

Rf value = Distance travelled by the center of the solute from origin / distance travelled by center of the solvent from origin.

Extraction of plant material: The bark was collected and cut into small pieces. The small pieces were dried at room temperature for about 2 days. Then they were dried in a hot air oven at a temperature 50 to 60°C for 5 to 6 h. Dried sample was crushed by electric grinder and coarse powder was passed through sieve no. 40. The sieved powder was subjected to extraction by taking hexane, methanol and water as solvents successively using soxhlet apparatus. Then the extract was dried to remove any moisture content present. The product was stored in the refrigerator maintaining a temperature 4°C.

Extraction procedure: About 50 g of dried powder was extracted with 150 ml hexane, 140 ml methanol and 100 ml water respectively. Extracts were concentrated, weighed and amount of yield was noted. The concentrated product was weighed and yield value was calculated. The three different extracts were coded as hexane extract (product code A), methanolic extract (product code B) and aqueous extract (product code C).

Preparation of ash

Muffle furnace: The fresh bark of *Ficus religiosa* was placed at muffle furnace maintained a temperature 450°C for 3 to 4 h. The product code was 'D'.

Direct incineration: The fresh bark of *Ficus religiosa* was collected. Direct fire or flame was applied until the bark became completed ash. The product code was 'E'.

Phytochemical screening of extract: The test was performed to identify the main phytoconstituents from different chemical constituents present in different extracts of Ficus religios by their colour reactions with different reagents. Each extract was subjected for phytochemical studies of glycosides (anthraquinone glycoside and cardio-glycoside), alkaloids, terpenoids, steroids, flavonoids, reducing sugars, tannins and saponins.

Antibacterial activities screening of extracts: Preliminary antibacterial test of *Ficus religiosa* extracts were carried out by cup diffusion method using Gentamycin sulphate (100µgm/ml) as standard.

Microorganisms, control and standard Gentamicin were obtained from Microbiology Laboratory of Sunsari Technical College.

Anti-microbial activities were performed in the bark extract product A, B, C, bark ash muffle furnace (D) and direct incineration (E) of the plant make: 800µgm/ml, 400µgm/ml, 200µgm/ml, 100 µgm/ml, 50 µgm/ml, concentration of stem bark extract, extracts and ash were prepared by dissolving in DMSO.

Methods

Antimicrobial test of plant extracts and ash were carried out by cup plate method.

Procedure

Muller Hinton Agar media was prepared and the media was sterilized in an autoclave at 121°C for about 15-20 min. The hot sterilized media was then cooled at 50-55°C. After cooling, the media was poured into cleaned and sterile petri plates of size 90 mm diameter such as each plate contained 20-25 ml of medium. The plates were allowed to cool for 20-25 min and got solidified. Bacterial suspension was prepared by inoculating loop full of bacteria in Brain Heart Infusion (BHI). Cups were made in agar plates with the aid of sterile cork borer of diameter 6mm and labeled properly.

The pure form of bacterial suspension was swabbed on the media with a sterile cotton swab in sterile condition and allowed to dry. To the different cups, 100µml of 800µg/ml, 400µg/ml, 200µg/ml, 100µg/ml, 50µg/ml concentration of each extract and ash of different dilution was placed with the help of micropipette. Also the standard drug was placed as Gentamicin and control (DMSO). All the plates were incubated at 37°C for 24 h followed by measuring zone of inhibition.

Result and Discussion

Macroscopic studies

Outer part of bark: The outer surface is brown or ash coloured with rough surface.

Inner part of bark: The inner surface is smooth and brownish specks and exfoliating in irregular rounded flakes.

Organoleptic properties: Colour: Bark is light gray and peels in patche, outer surface brown or ash colored, surface uneven due to exfoliation of cork. Inner surface was smooth and brownish in color.

Odor: Characteristics

Taste: Astringent

Plants extractive value

The yield value for each extract was determined. Nature of the extracts was mentioned in Table 1.

Sr.No.	Solvent used	Weight of yield (gm)	Nature of product
1	Hexane	3.15	Black and bit sticky
2	Methanol	2.77	Brownish crystalline
3	Water	2.59	Brownish crystalline

Table 1: Yield value of different extracts and their nature.

Total ash value: Total ash value was found to be 0.1566 gm and the percentage yield of ash was found to be 7.83%.

Phytochemical screening: Phytochemical screening of the plant revealed the presence of different constituents in different solvent extracts. The phytochemical analysis showed that the various groups

that were found to be present in the different extracts are listed in Table 2.

Phytochemicals	Plant extracts		
	Product A	Product B	Product C
Alkaloids	+	+	+
Glycosides	+	+	+
Saponins	+	+	-
Carbohydrates	+	-	+
Phenols	+	+	+
Flavonoids	+	+	-
Diterpines	+	+	+
Tannins	+	+	+
Proteins	+	+	-

Table 2: Phytochemical screening of plant extract.

'+' and '-' denotes the presence and absence of particular phytoconstituents, respectively

Phytochemicals screening of aqueous and methanolic extracts of *Ficus religiosa* bark showed the presence of tannins, saponins, flavonoids, terpenoids and steroids. It was reported that tannins, saponins, flavonoid, terpenoid are present in F. religiosa leaf and F. benghlensis leaf [9].

Loss on drying

- Initial Weight (Weight of petri dish + powder before drying) =100 g
- Final Weight (Weight of petri dish + powder after drying in oven) = 90.1 g
- Therefore, Loss on drying = (Weight of empty desiccator + Sample weight – Weight after drying)/ Sample weight = (90+ 10 – 99.01) / 10 = 0.099 g
- Total weight loss = 10 - 9.01 = 0.99 g
- Percentage loss on drying = (0.99/10) × 100 = 9.9 % w/w

Thin layer chromatography (TLC): Rf value of different extracts obtained from thin layer chromatography was given in Table 3.

Bark extract	Solvent used	Rf value
Product A	Hexane	0.8
Product B	Methanol	0.6
Product C	Water	0.7

Table 3: Rf value of different extracts performing tlc

Antibacterial activity: The antibacterial activity of the extracts and ash were evaluated based on the inhibition zone using the cup plate diffusion method (Table 4 & 5). The antibacterial activities suggest that the bark ash (product D & E) were more active than that of those three solvent extracts in terms of exhibiting antibacterial activities as they exhibited higher zone of inhibition in respect to all other extracts.

One thing common in each sample is that all the products (A to E) are significantly active against E.coli than S. aureus. But more or less those three solvent extracts also exhibited antibacterial activity which is due to presence of phytoconstituents specially like saponins, tannins, terpinoids [10-12] or due to synergistic effects of all the combined phytoconstituents.

Dilutions	Product A	Product B	Product C	Product D	Product E
Gentamycin	24±0.89*	25±0.87*	25±0.88*	24±0.34*	20±0.43*
100µg/ml					
800 µg/ml	10±0.23*	11±0.75*	11±1.02	14±0.34	13±0.67
400 µg/ml	08±0.67*	10±0.88	9±0.78	13±0.34	12±0.78*

200 µg/ml	6±0.87	7±0.86	8±0.89*	12±0.76	10±0.96
100 µg/ml	4±0.22	4±0.96	7±0.32	8±0.78	8±0.77*
50 µg/ml	-	-	-	7±0.54*	-
Control	-	-	-	-	-
DMSO					

Table 4: Zone of inhibition of different extracts and ash against s. aureus.

Dilutions	Product A	Product B	Product C	Product D	Product E
Gentamycin	24 ±0.63	24±0.62	26±0.86*	26±0.98	22±0.23
100µg/ml					
800 µg/ml	07±0.35	08±0.53	9±0.75	11±1.12	12±0.45
400 µg/ml	06±0.63*	05±0.68*	8±0.56*	10±0.78	11±0.34
200 µg/ml	05±0.72	04±1.04	7±0.67	9±1.08*	11±0.81*
100 µg/ml	04±1.04	03±0.34	4±0.43	7±0.67	10±0.34
50 µg/ml	-	-	-	5±0.32	7±0.87
Control	-	-	-	-	-
DMSO					

Table 5: Zone of inhibition of different extracts and ash against e. coli

Results were expressed as mean value ± standard error of the mean (SEM) of growth inhibition zones diameters. P values lower than 0.05 ($p<0.05$)* were considered significant.

Conclusion

Here detailed phytochemical, physiochemical studies of plant extract of different solvents and antibacterial activities of different solvent extract as well as for bark ash of *Ficus religiosa* Linn. (Moraceae) was carried out. The result of present study signifies that the bark ash of this plant is found much more effective as an antibacterial agent in comparison to other solvent extracts of this plants as a result this study suggests that this plant ash can be used as a source for formulation of antibacterial agents.

This study lastly concludes that even nowadays the traditional therapy using bark ash for bacterial infections is worth better than any other formulated drugs especially in rural areas of Nepal.

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