PHYTOCHEMICAL SCREENING, ANTIMICROBIAL AND ANTIDIARRHEAL POTENTIAL OF *BRIDELIA VERRUCOSA* HAINES

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خلاصہ

یہ مطالعہ بریڈیلیاورروکوساہنیز کے ایتھنلوک چھال اور پتی کے عرق کی فائیٹوکیمیکل، اینٹی بیکٹیریل اور اینٹیڈ بیئرال صلاحیت کی تحقیقات کے لئے کیا گیا تھا۔ فائٹوکیمیکل تجزیہ کے نتائج نے دونوں نچوڑوں میں الکلائڈ، فلاوونائڈز، ٹینئز، سٹیرایڈاور سیپونن کی موجودگی کو ظاہر کیا۔ بی ورروکوساکے چھال کے عرق نے پتی کے عرق کے مقابلے میں فلاونائڈز، سیپونئزاور ٹیرپینائڈز کی زیادہ مقدار ظاہر کی۔ آگر اچھی طرح سے پھیلاؤپر وٹوکول گرام مثبت (سٹیفیلوکوکس اوریئس اور بیسیلس سبٹیلیس) اور گرام منفی (ایکینیٹوبایکٹر بومنینی، ایسچریچیاکولی اور کلیبسیلانمونیہ) کے خلاف بی وریروکوساکے اینٹی بیکٹیریل صلاحیت کا اندازہ کرنے کے لئے استعمال کیا گیا تھا۔ چھال کے نچوڑنے بہتر اینٹی بیکٹیریل صلاحیتوں کی نمائش کی۔ یعنی 263 ± 0.65ملی میٹر، 18 ± 0.8ملی میٹر ایکینیٹوبایکٹر بومنی اور ایشیریچیاکولی کے خلاف اور سب سے کم زون یعنی 11 0.3 bsملی میٹر کلیبسیلانمونیا کے خلاف دیکھا گیا۔ نتائج کا موازنہ کرنے کے لئے اسٹریپٹومیسن اور ٹیٹراسائکلین ایک معیاری ادویات کے طور پر استعمال کی گئیں۔ معیاری اینٹی بائیوٹک ادویہ یعنی ٹیٹراسائکلین نے اسٹریپٹومیسن کے مقابلہ میں بہترین نتائج دکھائے۔ اینٹیڈیریریل سرگرمی کا اندازہ ارنڈی تیل کی حوصلہ افزائی اسہال کے ذریعے کیا گیا تھا۔ اسہال کے قطرے کے اخراج میں نمایاں کمی 350اور 550ملی گرام/ کلو گرام ٹریٹمنٹ اقتباس کے ذریعہ تیار کی گئی تھی۔ برڈیلیاورریکو چوپتیوں نے اسہال کے خلاف 350ملی گرام/ کلو گرام خوراک کے ساتھ تسلی بخش نتائج ظاہر کیے جبکہ 550مگرا/ کلو گرام کے ساتھ کم سے کم امکان ظاہر کیا۔ جبکہ چھال کی دونوں خوراکیں اسہال میں نمایاں کمی کو پیش کرتی ہیں جس میں قابلیت کے ساتھ مثبت قابلیت لوپیرمائڈ کے ساتھ دونوں خوراکوں کے ساتھ ہیں۔ گیلے ملا کے فیصد فیصد روکنے کے اہم نتائج چھال کے نچوڑ میں 1، 2، 3اور 4 بجے 26.3٪، 45٪، 59.5٪اور 64.2 فیصد کے طور پر دیکھے گئے۔ ان نتائج سے یہ انکشاف ہوا ہے کہ پتی کے عرق کے مقابلے میں بی ورروکوساکے چھلنے کے اعداد و شمار نے اینٹی مائکروبیل اور انسداد اسہال کی سرگرمیوں کی طرف زیادہ صلاحیت ظاہر کی ہے۔

Abstract

The study was carried out to investigate the phytochemical, antibacterial and antidiarrheal potential of the ethanloic bark and leaf extract of *Bridelia verrucosa* Haines. The results of phytochemical analysis showed the presence of alkaloid, flavonoids, tannins, steroid and saponins in both extracts. The bark extract of *B. verrucosa* showed the higher amount of flavonoids, saponins and terpenoids *as* compared to leaf extract. Agar-well diffusion protocol was used to evaluate the anti-bacterial potential of *B. verrucosa* against Gram Positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella pneumonia*). The bark extract exhibited better antibacterial potential i.e.23 ±0.65mm, 18± 0.8mm against *Acinetobacter baumannii* and *Escherichia coli* and the lowest zone i.e. 11±0.3 mm was observed against *Klebsiella pneumonia*. The streptomycin and tetracycline were used as standard drugs to compare the results. The standard antibiotic drug i.e. tetracycline showed best results as compared to streptomycin. The Antidiarrheal activity was evaluated through Castor-oil induced diarrhea. Significant reduction in the excretion of diarrheal drop was produced by the treatment extract of 350 and 550 mg/kg. *B. verrucosa* leaves showed satisfactory results with 350 mg/kg dose against diarrhea and least potential with 550 mg/kg. Whereas the both doses of bark presents significant reduction in diarrhea with both doses with competence to positive control loperamide. The significant results of Percentage inhibition of wet feces were observed in bark extract as 26.3%, 45%, 59.5% and 64.2% at 1, 2, 3 and 4 hours. The results revealed that bark extract of *B. verrucosa* showed higher potential towards Anti-microbial and Anti-diarrheal activities as compared to leaf extract.

Keywords: Phytochemical screening, antibacterial activity, *Bridelia verrucosa* Haines.

Introduction

For thousands of years' plants have been used for health and medicinal purpose and provides a new way for the development of modern drugs. Plants play a major part in the biodiversity on the earth and they are also rich resources of component which can be used in drug development (Ajaib et al., 2021a). These herbal resources can be used in synthesis of medicinal plant such as blood thinners, antibiotics and anti-malaria medications (Aggarwal, 2003).

The multidrug resistant strain developed in pathogenic bacteria have become hazard for the health of people and there are no significant antimicrobial agents existing for antibacterial infection. Antimicrobial agents play an important role against infectious diseases (Giamarellou, 2010). Natural antimicrobial compounds have been recognized from different medicinal plants that were potentially more active for bacterial infections due to the presence of secondary metabolites which have their antimicrobial potential (Ajaib *et al.,* 2016; Romero *et al.,* 2005).

The World Health Organization, reported that variety of drugs have been obtained from medicinal plants (WHO, 2002). Plants have variety of compounds such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been used *in vitro* as for antimicrobial potential and for treating infectious diseases as cutaneous infections, gastrointestinal disorders and respiratory disease (Duraipandiyan *et al*., 2006).

Diarrhea is characterized by the increase level of secretion and motility in gastric intestine which result in decrease absorption of electrolytes and fluid (Kelechi *et al*., 2012). Diarrheal disease lead to the death of infants and children in developing countries and 2.5 billion cases of diarrheal patients are reported every year around the world in which death rate of 1.9 million children are recorded below the age of 5 years (WHO, 2010).

Bridelia verrucosa Haines is a large shrub without spikes and commonly known as Ghiyai or Chilla which belong to family Phyllanthaceae. The phytochemical screening of leaf extract of *Bridelia verrucosa* showed the presences of different compounds such as glucoside, sitosterol and hexacosanol due to which they have been used for the treatment of different diseases and known for anthelmintic potential (Ajaib *et al.,* 2021b).

Material and Method

Collection of Plant Material: The bark and leaf of *Bridelia verrucosa* Haines were collected from different regions of District Bhimber, Azad Jammu and Kashmir, Pakistan. The collected plant material was identified and authenticated (Herbarium Department of Botany, MUST, voucher number of MUST.BOT.5380).

Plant Material: The plant material of *Bridelia verrucosa* are parted into its constituents, such as bark and leaf scattered evenly on newspaper and placed in the shade for drying at room temperature until plants part dried completely. The fully dried up leave and bark of plant was ground to powder form and stored in air tight jar.

Preparation plant Extract: Plant powder of 250g bark and leaf of *Bridelia verrucosa* Haines were weighed and soaked in 1000 ml of ethanol for 7 days and was shaken at different time interval for proper extraction. After 7 days the filtrate was filtered through Whatmann filter paper. This method was repeated twice or thrice to completely exhaust the plant material. After that, the filtrate was solidified using rotatory evaporator at 35-40˚C under reduced pressure. Finally, extract of the plant was obtained and stored at -4˚C.

Phytochemical Analysis: The qualitative phytochemical analysis was carried out through specific methods of Evans and Trease, (2009) to determine the presence or absence of alkaloids tannins, flavonoids, saponins, phenols, terpenoids, steroids, glycosides, and proteins in the ethanolic bark and leaf extract of *Bridelia verrucosa* Haines.

Antibacterial Activity: The antibacterial activity of bark and leaf extract of *Bridelia verrucosa* Haines was carried out by using Agar well diffusion method of Jorgenson (2007) and Ajaib *et al*., (2016).

Sub-culturing of Organism: The cultures of the selected microorganisms were streaked onto Nutrient agar (NA) plates inside the laminar flow chamber which was then incubated for 24 hours at 37ºC before use (Romulo *et al*., 2018).

Microorganisms: The four bacterial strains were tested in this study which included Gram Positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*A. baumannii, E. coli* and *K. pneumonia*) obtained from Department of Microbiology, G.C.U, Lahore.

Antidiarrheal Activity

Experimental Animals: The albino mice of same sex were used for the experiment. The mice were kept with due permission of ethical committee of G.C.U, Lahore in the Zoology, Department. The age of mice was about 6 to 8 week and the weight was about 20 to 30g. The animals were placed in plastic cages at 25 ± 3˚C and 12v hour light/dark cycle. Subtraction of feces from cages and constant cleaning thrice a week was performed. The mice were adapted to laboratory situations for 1 week before to the experiment. For the use and attention of experimental animals, care and handling was maintained according to international guidelines.

Ethics Approval and Consent to Participate: All the adopted procedure were approved by Animal Ethical Committee of G.C.U, Lahore in the Zoology, Department.

Human and Animal Rights: No humans were used in this research. All experiments were performed in accordance with the "European Convention for the protection of Vertebral Animals used for Experimental and other Scientific purpose," 1986.

Grouping and Dosing of Animals: For the evaluation of antidiarrheal activity, 30 mice were used which were divided in to 6 groups. The negative control groups were treated with the10 ml/kg of distilled water and 0.5ml castor oil. The positive controls were treated with 3mg/kg of loperamide. The plant treatment group were treated with 350, and 550 mg/kg doses of extract.

Castor Oil-induced Diarrhea: For the evaluation of antidiarrheal activity of ethanolic leaf and bark extract of *B. verrucosa*, the model of castor oil induced diarrhea of Lorke (1983) was used. The % inhibition of excretion in mice was calculated by following the method of Diurno *et al*. (1996).

$$\% \text{ Inhibition} = \frac{(MC - MT)}{MC \times 100}$$

MC = Mean defecation of control
MT = Mean defecation of test groups

Results and Discussion

Phytochemical Screening: Phyto chemicals are produced mainly by plants, and these are biologically active compounds. The Phytochemical screening of the ethanolic leaf and bark extract was accomplished qualitatively for the estimation of compounds in plant material. Several tests were performed to investigate the presence or absence of compounds i.e. Carbohydrates, flavonoids, terpenoids, tannins, alkaloids, saponins glycosides and proteins. (Table-1). Both the leaf and bark extracts of *B. verrucosa* showed the presence of bioactive compound. The bark extract of *B. verrucosa* showed the higher amount of flavonoids, saponins and terpenoids *as* compared to leaf extract.

Tabel 1. Phytochemical analysis leaf and bark extract of *B. verrucosa*

Plant parts	Solvent	Phytochemicals								
		Alka--loids	Carbo--hydrates	Flavo--noids	Glyco--sides	Proteins	Steroids	Saponins	Tannins	Terpe--noids
Leaf	Ethanol	+	+	+	+	++	+	+	+	+
Bark		++	+	+++	+	++	++	+++	++	+++

+ = Minute quantity, ++ = Moderately high concentration +++ = Very high concentration

Antibacterial activity: Antibacterial study was carried out against bacterial strains and the negative control was established for evaluating the extracts of plant against strains. In bacterial activity both extract of bark and leaf of *Bridelia verrucosa* showed activity against all bacterial strains but the ethanolic bark extract revealed better results. The results for antibacterial activity of ethanolic leaf extracts revealed that the highest zone of inhibition i.e. 16±0.3 mm was produced against *B. subtilis* and the lowest zone of inhibition i.e. 10.3±0.3mm against *K. pneumoniae*. Whereas the bark extract exhibited better antibacterial potential i.e.23 ±0.65mm, 18± 0.8mm against *A. bauminii* and *E. coli* and the lowest zone i.e. 11±0.3 mm was observed against *K. pneumoniaee*. The different standard drugs such as streptomycin and tetracycline were employed against all bacterial strains and zone of inhibitions produced by them were also recorded to compare the results. The standard antibiotic drug i.e. tetracycline showed best results as compared to streptomycin (Table-2, Fig-1).

Tabel 2. Antibacterial activity of leaf and bark of *Bridelia verrucosa* against bacterial strains and antibiotics

Plant parts	Solvent	Bacterial strains				
		Gram positive		Gram negative		
		S. aureus	*B. subtilis*	*A. baumannii*	*E. coli*	*K. pneumoniae*
Leaf	Ethanol	14±0.8	16±0.5	15.3±0.6	15±0.5	10±0.3
Bark		17±0.5	18±0.8	23±0.5	19±0.6	11±0.3
Antibiotic						
Streptomycin		12±0.5	13±0.8	16±0.3	14±0.5	9±0.3
Tetracycline		15.±0.3	17.±0.8	21±0.3	18±0.5	11±0.5

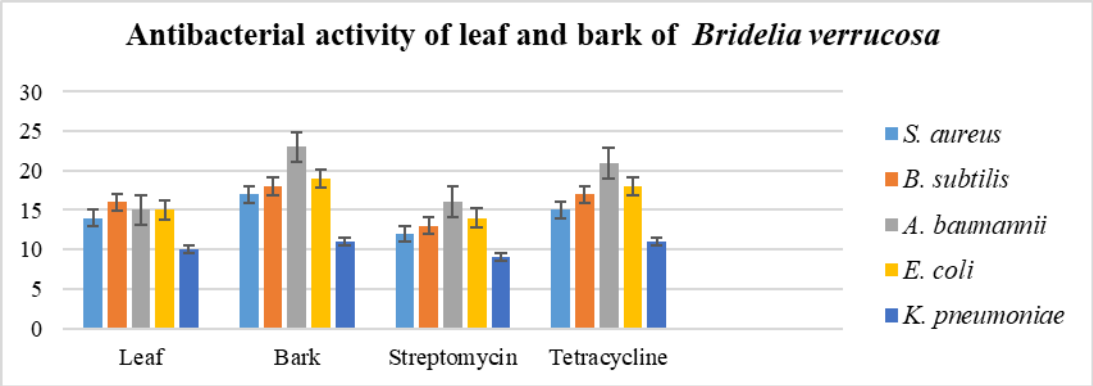

Fig-1 Antibacterial activity of leaf and bark of *Bridelia verrucosa*

Anti-diarrheal activity evaluation

In the castor oil-induced diarrhea experiment, the leaf and bark extract of *Bridelia verrucosa* Haines produced a marked antidiarrheal effect in mice. The dose of 350mg/kg and 550 mg/kg of both leaf and bark extracts were used to analyze the anti-diarrheal effect. All the four doses of both extracts did not show any adequate response against diarrhea in 1 hr. The treatment group of leaf extract (350 mg/kg) showed weak resistance against diarrhea in 2h with 124.8±1.49, followed by promising anti-diarrheal effect in 3 and 4h with 86.4±2.80 and 68±4.56 respectively. The treatment group of bark extract showed efficient results against diarrhea with both doses (350 and 550 mg/kg). The 350 mg/kg dose proceeded anti-diarrheal effect in 1h, 2h, 3h and 4h as 120.2±2.45, 105±1.94, 95.6±0.92 and 74.4±1.07 respectively. The 550 mg bark showed 116±2.12, 93±1.87, 77.8±3.27 and 62.2±1.46 in 1h upto 4h respectively, with significant reduction in diarrhea (Table-3, Fig-2). All the results indicated that the bark extract of *B. verrucosa* were more effective against diarrhea as compared to leaf extract.

Table 3. Antidiarrheal activity of leaf and bark extract of *B. verruccosa*

Treatment groups	Dose	Mean weight of fecal discharge			
		1h	2h	3h	4h
Normal saline	10ml/kg	125.6±2.01	136.8±1.2	158±±0.7	166.6±1.8
Loperamide	5mg/kg	91.9±2.11	75.2±1.65	64.2±1.71	59.54±2.60
Leaf	350 mg/kg	115.4±3.32	124.8±1.49	86.4±2.80	68±4.56
	550 mg/kg	118.4±1.16	133.8±1.39	152.8±3.33	136.8±3.29
Bark	350 mg/kg	120.2±2.45	105±1.94	95.6±0.92	74.4±1.07
	550 mg/kg	116±2.12	93±1.87	77.8±3.27	62.2±1.46

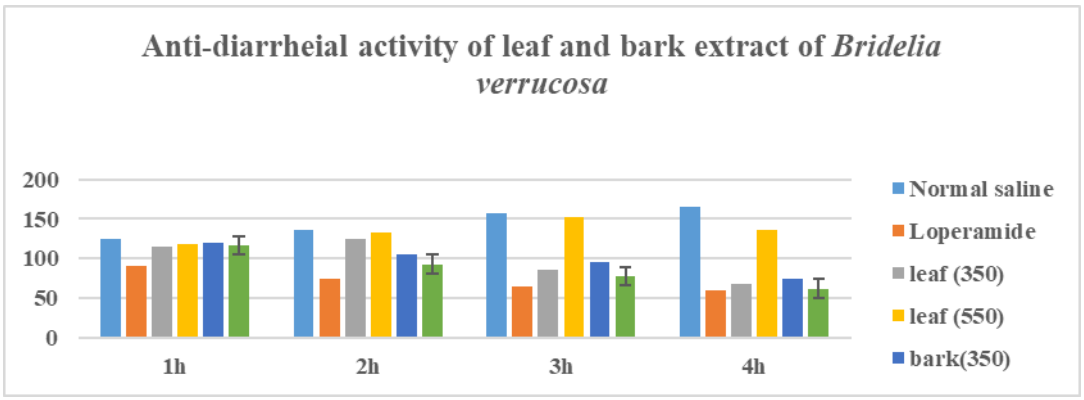

***Fig. 2. Anti-diarrheal activity of leaf and bark extract of* Bridelia verrucosa**

The percentage inhibition was found to become more prominent as from 1h to 4h in leaf 350 mg/kg, i.e. 8.1%, 8.77%, 45.5% and 59.1 % respectively while least inhibition in 550 mg/kg was 5.73%, 2.19%. 3.63% and 17.8% from 1h to 4h manually. More significant inhibition was seen with bark 550 mg/kg with 7.6%, 32%, 50.9% and 62.6% following 1h to 4h respectively. 350 mg/kg bark showed percentage inhibition from 1-4h as 4.4%, 23.2%, 39.7% and 55.3% respectively. The percentage inhibition was compared with positive control loperamide, having values 26.3% in 1 h, 45% in 2h, 59.5% in 3h and 64.2% in last hour (Table-4, Fig-3).

Table 4. Percentage Inhibition of leaf and bark extract of *Bridelia verrucosa* Haines

Groups	Doses (mg/kg)	% inhibition			
		1h	2h	3h	4h
Normal saline	10	-	-	-	-
Loperamide	5	26.3	45	59.5	64.2
Leaf	350	8.1	8.77	45.5	59.1
	550	5.73	2.19	3.63	17.8
Bark	350	4.4	23.2	39.7	55.3
	550	7.6	32	50.9	62.6

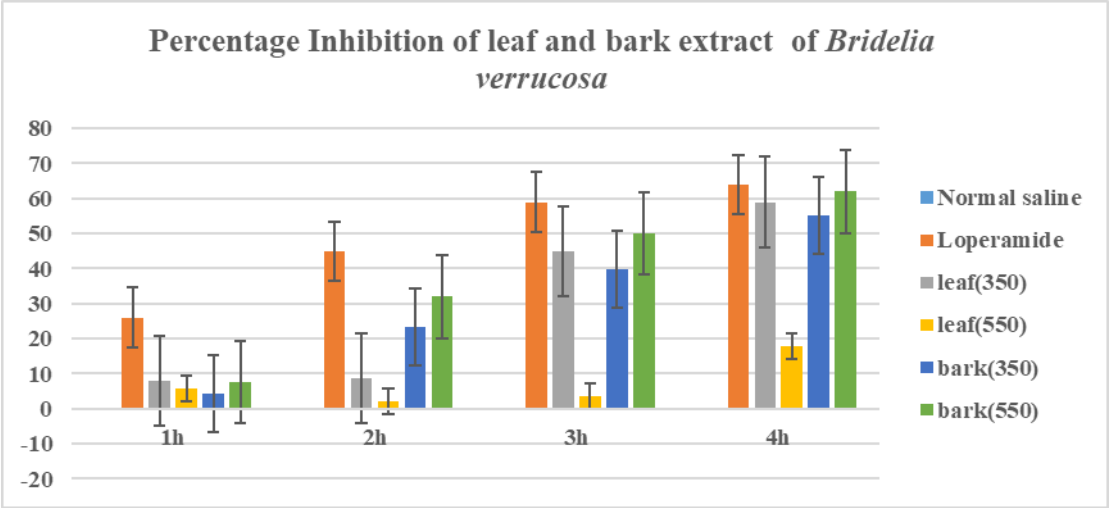

Fig-3 Percentage Inhibition of leaf and bark extract of *Bridelia verrucosa*

The results of antibacterial activity revealed the importance of plant extract when compared with antibiotics that showed resistance against bacteria at low concentration. Both extract of bark and leaf of *B. verrucosa* Haines showed activity against all bacterial strains but the ethanolic bark extract revealed better results. The similar results were reported by Cheruiyot *et al*. (2009) working on antimicrobial activities of methanolic leaf extracts of *Psidium guajava* against *P. aeruginosa*, *E. coli* and *S. aureus*.

The study was conducted to evaluate the antidiarrheal effect of leaf and bark extract of *B. verrucosa* in mice by using antidiarrheal activity test model. The castor oil-induced diarrhea showed that doses of both leaf and bark extract significantly reduced the intestinal fluid when compared with the negative controls. When concentration of plant extract increased, maximum reduction in intestinal content was observed. The plant extract showed better results when compared with the loperamide a standard drug. The results of the study showed close resemblance to the work of Mekonnen *et al*. (2018) evaluating the antidiarrheal activity of 80% methanolic leaf extract of *Justicia schimperiana.*

Conclusion

It is concluded that the bark extract of *B. verrucosa* showed higher potential to wards Anti-microbial and Anti-diarrheal activities as compared to leaf extract due to the presence of rich amount of secondary metabolites.

Acknowledgment

The authors are thankful to Department of Zoology GC University Lahore for providing laboratory facilities.

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