

**EVALUATION OF *ARISTOLOCHIA BRACTEOLATA* LINN. FOR ANTIMICROBIAL ACTIVITY,  $\alpha$ -GLUCOSIDASE INHIBITION, AND ITS PHYTOCHEMICAL CONSTITUENTS**

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**ABSTRACT****Objective:** To evaluate the antimicrobial efficacy,  $\alpha$ -glucosidase inhibition, and various phytochemical constituents of *Aristolochia bracteolata* Linn.**Methods:** Soxhlet extraction using hexane, ethyl acetate, and methanol solvents, antimicrobial,  $\alpha$ -glucosidase inhibition assay, qualitative and quantitative phytochemical estimation.**Results:** The results revealed that the methanolic extract showed significant antibacterial and antifungal activity. The maximum zone of inhibition was against *Bacillus subtilis* (24 mm), *Yersinia enterocolitica* (22 mm), and *Proteus vulgaris* (20 mm) at the concentration of 5 mg/ml of extract. Methanolic extract showed minimum inhibitory concentration value of 250  $\mu$ g/ml against all the fungal pathogens. The methanol extract showed 78.27% of  $\alpha$ -glucosidase inhibition. The phytochemical tests showed the presence of phenols, terpenoids, tannins, flavonoids, glycosides, carbohydrates in methanol extract. Total phenolic and total flavonoid content of methanol extract were 103.21 $\pm$ 1.23 mg catechol equivalents/100 g extract and 53.01 $\pm$ 1.78 mg quercetin equivalents/100 g, respectively.**Conclusion:** The results conclude that the extracts of *A. bracteolata* exert multiple biological properties due to the presence of flavonoids and phenols. It is also an effective glucosidase inhibitor. It can be used to obtain novel antibacterial compounds for the treatment of infectious diseases in future.**Keywords:** *Aristolochia bracteolata* Linn., Phytochemical, Phenolic and flavonoid content, Antimicrobial activity,  $\alpha$ -glucosidase inhibition.**INTRODUCTION**

India has one of the oldest, richest, and most diverse cultural traditions associated with the use of medicinal plants. Medicinal plants have provided the basic building blocks for a number of highly effective drugs [1]. The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents [2]. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables, and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Medicinal plants play a vital role in preventing various diseases. The antidiuretic, anti-inflammatory, antianalgesic, anticancer, antiviral, antimalarial, antibacterial, and antifungal activities of the medicinal plants are due to the presence of the above-mentioned secondary metabolites. Medicinal plants are used for discovering and screening of the phytochemical constituents which are very much helpful for the manufacturing of new drugs. Medicinal plants are the richest bioresource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, and chemical entities for synthetic drugs [3]. Plants are endowed with various phytochemical molecules such as vitamins, terpenoids, phenolic acids, lignin, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, and other metabolites, which are rich in antioxidant activity [4,5].

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for

new and re-emerging infectious diseases [6,7]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [8]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents hassled to the screening of several medicinal plants for their potential antimicrobial activity [9-11].

In India, it is declared that traditional healers use 2500 plant species, and 100 species of plants serve as natural principles of medicine [12]. With a view to increasing the wide range of medicinal usages, the present day entails new drugs with more potent and desired activity with less or no side effects against particular disease [13]. *Aristolochia* is an important genus in the family of Aristolochiaceae. The genus *Aristolochia* consists of about 400 species of herbaceous perennials, under shrubs or shrubs bearing essential oils and is widespread across tropical Asia, Africa, and South America [14]. *Aristolochia* species has been used extensively in the traditional Chinese medicine. Its diverse biological functions include hypertension relief, leukocyte enhancement, rheumatism relief, edema therapy, as well as analgesic and diuretic effects [15-18]. Various *Aristolochia* species have been used in herbal medicines since antiquity in obstetrics and in the treatment of snakebite [19], festering wounds, and tumors and they remain in use particularly in Chinese herbal medicine [20]. *Aristolochia bracteolata* Linn. (Aaduthinnapaalai-Tamil; Worm killer-English; Gadaparku-Telugu; Bhringi-Hindi) is a shrub distributed throughout India. In the indigenous system of medicine, the plant was used for the treatment of skin diseases, inflammation, and purgative [21]. Root extract was reported to have antibacterial activity [22] and also the toxicity of *A. bracteolata* was reported by Harborne [23]. The leaves of this plant are mainly used by native tribal and the villagers. It is commonly called as "worm killer" in English and aaduthinnapaalai in Tamil, due to supposed anthelmintic activity and trypanocidal effect [24]. It is used in traditional medicines as a gastric stimulant and in the treatment of cancer, lung inflammation, dysentery,

and snakebites. Methanolic extract of plant parts of *A. bracteolata* was the source of physiological active compounds. The use of the plant as an antimalarial is not recommended in its crude form [25]. The whole plant was used as a purgative, antipyretic, and anti-inflammatory. It also possesses a potent antiallergic activity [26] and has pronounced antibacterial and antifungal activities [27]. The present paper aims to study the antimicrobial activity and  $\alpha$ -glucosidase inhibition tests on the solvent extracts of *A. bracteolata*.

### Ethno botanical survey [28]

#### Scientific classification

Kingdom: Plantae  
Order: Piperales  
Family: Aristolochiaceae  
Subfamily: Aristolochioideae  
Genus: *Aristolochia*  
Species: *bracteolata*

### METHODS

#### Collection of plant material

Plants were obtained from places near Coimbatore, South India. The plant specimen *A. bracteolata* was identified, and the voucher specimen number (LCH 310) was deposited at Loyola college herbarium in the Department of Plant Biology and Biotechnology, Chennai, Tamil Nadu, India.

#### Preparation of the extract

The solvents which were used for the preparation of extract were hexane, ethyl acetate, and methanol. 10 g of whole plant powder was taken, and the extract was prepared with Soxhlet using 100 ml of each solvent according to their polarities starting with a solvent of low polarity to high polarity. The extract was then filtered using membrane filter (0.45  $\mu$ m). The obtained filtrate were concentrated under reduced pressure at 40°C using rotary evaporator and stored in a refrigerator at 2-8°C for use in subsequent experiments.

#### Qualitative phytochemical analysis

Phytochemical analysis of *A. bracteolata* was done with respect to all above mentioned extracts. All the crude extracts were analyzed for the presence of alkaloids, saponins, protein, coumarins, phenols, triterpenes, steroids, flavonoids, and tannins according to standard methods of Harborne [29].

#### Quantitative phytochemical analysis

##### Determination of total phenolic content

Total phenolic content was assessed according to the Folin-Ciocalteu method of Slinkard and Singleton [30] with some modifications. Briefly, 0.1 ml of sample (200–1000  $\mu$ g/ml), 1.9 ml distilled water, and 1 ml of Folin-Ciocalteu's reagent were seeded in a tube, and then 1 ml of 100 g/l  $\text{Na}_2\text{CO}_3$  was added. The reaction mixture was incubated at 25°C for 2 hrs, and the absorbance of the mixture was read at 765 nm. The sample was tested in triplicate, and a calibration curve with six data points for catechol was obtained. The results were compared with catechol calibration curve, and the total phenolic content of *A. bracteolata* extracts were expressed as mg of catechol equivalents per 100 g of extract.

##### Determination of total flavonoid content

Total flavonoid contents of *A. bracteolata* were determined by the aluminum chloride colorimetric method as described by Willet [31] with some modifications and reported as Quercetin Equivalents (QE) per 100 g of extract. 0.5 ml of each hexane, ethyl acetate, and methanol extracts, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 4.3 ml distilled water were added to each test tube. All the tubes were incubated at room temperature for 30 minutes. Absorbance was measured at 415 nm using UV spectrophotometer (UV-1800, Shimadzu). Quercetin was used to make a calibration curve. The calculation of total flavonoids in the extracts was carried out in triplicates, and the mean values were represented.

#### Antibacterial activity

All the microbial strains used in the antimicrobial bioassay were procured from Institute of Microbial Technology, Chandigarh, India. *Micrococcus luteus* (MTCC 106), *Enterococcus faecalis* (ATCC 29212), *Vibrio parahaemolyticus* (MTCC 451), *Pseudomonas aeruginosa* (ATCC 27853), *Proteus vulgaris* (MTCC 1771), *Enterobacter aerogenes* (MTCC 111), *Staphylococcus aureus* (MTCC 96), *Klebsiella pneumoniae* (MTCC 109), *Staphylococcus epidermidis* (MTCC 3615), methicillin-resistant *S. aureus* (MRSA), *Bacillus subtilis* (MTCC 441), and *Yersinia enterocolitica* (MTCC 840). The antimicrobial screening against various given clinical pathogens was performed using the disc diffusion method followed by Latha et al. [32]. Petri plates were prepared with 20 ml of sterile Mueller-Hinton Agar (MHA) (Hi-media, Mumbai). The test culture (100  $\mu$ l of a suspension containing  $10^8$  CFU/ml bacteria) were swabbed and allowed to dry for 10 min. The discs were each impregnated with 20  $\mu$ l of extract (10 mg/ml) at different concentrations of (5, 2.5 and 1.25 mg/disc) and placed on the medium and incubated at 37°C for 24 hrs. Reference antibiotic streptomycin 10  $\mu$ g/disc was used.

#### Antifungal activity

All the respective extracts were preliminary screened for antifungal activity against pathogenic fungal strains such as *Aspergillus flavus* (MTCC 10938), *Botrytis cinerea* (MTCC 2880), *Curvularia lunata* (MTCC 5109), *Aspergillus niger* (MTCC 1344), *Trichophyton rubrum* (MTCC 296), and *Trichophyton mentagrophytes* (MTCC 8476) using the standard reference method (NCCLS) [33]. The extracts were dissolved in water with 20% dimethyl sulfoxide. The initial concentration of the extract was 1 mg/ml. The initial test concentration was serially diluted 2-fold. Each well was inoculated with 5  $\mu$ l of fungal suspension and incubated at room temperature for 3 days. The antifungal agent fluconazole was used as a reference. MIC was defined as the lowest extract concentration, showing no visible fungal growth after incubation time.

#### Determination of $\alpha$ -glucosidase inhibition

##### $\alpha$ -glucosidase inhibition assay

In order to investigate the inhibition activity of hexane, ethyl acetate, and methanol extract of *A. bracteolata*, an *in vitro*  $\alpha$ -glucosidase inhibition test was performed.  $\alpha$ -glucosidase from yeast is used extensively as a screening material for  $\alpha$ -glucosidase inhibitors, but the results do not always agree with those obtained in mammals. Therefore, we used the mouse small intestine homogenate as  $\alpha$ -glucosidase solution, since we speculated that it would better reflect the *in vivo* state. The inhibitory effect was measured using the method slightly modified from Dahlqvist [34]. After fasting for 20 hrs, the small intestine between the part immediately below duodenum and the part immediately above the cecum was incised, rinsed with ice-cold saline, and homogenized with 12 ml of malate buffer (100 mM, pH 6.0). The homogenate was used as the  $\alpha$ -glucosidase solution. The assay mixture consisted of 100 mM malate buffer (pH 6.0), 2% (w/v) each sugar substrate solution (100  $\mu$ l), and the sample extract (200–1000  $\mu$ g/ml). It was pre-incubated for 5 minutes at 37°C. The reaction was initiated by adding the crude  $\alpha$ -glucosidase solution (50  $\mu$ l) to it, followed by incubation for 10 minutes at 37°C. The glucose released in the reaction mixture was determined with the kit described above. The rate of carbohydrate decomposition was calculated as the percentage ratio to the amount of glucose obtained when the carbohydrate was completely digested. The rate of inhibition was calculated by the following formula.

$$\text{Inhibition (\%)} = \frac{[(\text{amount of glucose produced by the positive control}) - (\text{amount of glucose produced by the addition of sample}) / (\text{amount of glucose produced by the positive control})] \times 100}{100}$$

### RESULTS

#### Phytochemical analysis

Phytochemicals are biologically active compounds presents in plants used for food and medicine. Phytochemical characteristics of the

leaf extract of *A. bracteolata* investigated are summarized in Table 1. Preliminary phytochemical study reveals the presence of glycosides, carbohydrate in all the three hexane, ethyl acetate and methanol extracts, presence of steroids in hexane ethyl acetate and methanol extract, presence of flavonoids and terpenoids in hexane and methanol extract, presence of tannins and phenol in methanol extract, presence of coumarins in hexane extract followed by absence of quinones, cardiac glycosides, anthraquinones, alkaloids, saponins, and protein. All the extracts were subjected to further analytical tests for the quantification of phytochemical constituents.

**Determination of total phenolic content**

Among the three extracts, methanol extract containing the highest 103.21±1.23 amount of phenolic compounds followed by ethyl acetate extract 60.16±1.72 and hexane 53.69±1.59 mg catechol equivalents/100 g of dry mass. The results were reported in Table 2.

**Determination of total flavonoid content**

The total flavonoid content of hexane, ethyl acetate, and methanol extracts of *A. bracteolata* were found to be 24.26±0.89, 43.97±1.28, and 53.01±1.78 mg QEs/100 g of dry mass, respectively. The results were reported in Table 2.

**Antimicrobial assay by disc diffusion method**

The antimicrobial activities of the plant were assayed *in vitro* by the agar disc diffusion method against ten bacterial pathogens. Table 3 summarizes the antibacterial activity where the methanol extract of *A. bracteolata* exhibits a significant zone of inhibition followed by ethyl acetate and hexane extract, respectively, against various test cultures. Methanol and ethyl acetate extract possess a significant zone of inhibition against *B. subtilis* (20, 22, 24 mm), (20, 22, 23 mm) followed by *Y. enterocolitica* (18, 20, 22 mm), (14, 15, 16 mm), *P. vulgaris* (16, 17, 20 mm), (16, 18, 19 mm), whereas hexane extract for the same pathogens showed no activity. Methanol extract shows activity against *E. faecalis* and *Staphylococcus epidermidis* (9, 10, 11 mm). Methanol, ethyl acetate, and hexane extract show significant zones against *E. aerogenes* (10, 12, 15 mm), (11, 12, 13 mm), (11, 12, 13 mm); *Micrococcus luteus* (9, 11, 12 mm), (11, 12, 13 mm), (8, 9, 10 mm); MRSA (9, 10, 11 mm), (8, 9, 10 mm), (8, 9, 10 mm); *S. aureus* (9, 10, 11 mm), respectively, in three different concentrations, i.e., 1.25, 2.5, and 5 mg/ml of extract.

**Table 1: Qualitative phytochemical analysis of *Aristolochia bracteolata* Linn.**

Phytochemicals	Hexane	Ethyl acetate	Methanol
Coumarins	+	-	-
Steroids	+	+	-
Quinones	-	-	-
Phenols	-	-	+
Terpenoids	+	-	+
Cardiac glycosides	-	-	-
Tannins	-	-	+
Anthraquinone	-	-	-
Flavonoids	+	-	+
Alkaloids	-	-	-
Glycosides	+	+	+
Saponins	-	-	-
Protein	-	-	-
Carbohydrates	+	+	+

+: Present, -: Absent

**Table 2: Total phenolic and flavonoid content of different solvent leaf extracts of *Aristolochia bracteolata* Linn.**

Phytochemicals	Hexane	Ethyl acetate	Methanol
TPC (CE/g) <sup>a</sup>	53.69±1.59	60.16±1.72	103.21±1.23
TFC (QE/g) <sup>b</sup>	24.26±0.89	43.97±1.28	53.01±1.78

<sup>a</sup>(mg CE/100 g of dry mass), C: Catechol, <sup>b</sup>(mg QE/100 g of dry mass),

Q: Quercetin. TPC: Total phenolic content, TFC: Total flavonoid content

*K. pneumoniae*, *P. aeruginosa*, and *V. parahaemolyticus* does not show any inhibition against the extracts of *A. bracteolata*.

**Minimum inhibitory concentration (MIC)**

Among the three extracts, methanol extract of *A. bracteolata* shows significant antifungal activity with the minimum concentration of 250 µg/ml against the pathogenic fungi namely *A. niger*, *B. cinerea*, *T. rubrum*, *C. lunata*, *A. flavus*, and *T. mentagrophytes*, whereas the ethyl acetate extract shows MIC value of 500 µg/ml against *A. niger*, *C. lunata*, *T. mentagrophytes*, 250 µg/ml against *B. cinerea*, *T. rubrum*, *A. flavus*, and hexane extract exhibits minimum inhibition with the concentration of 500 µg/ml against *A. niger*, *T. rubrum*, *C. lunata*, *A. flavus*, 250 µg/ml against *B. cinerea*, and *T. mentagrophytes*. The results were elucidated in Table 4.

**α-glucosidase inhibition efficacy**

The results of α-glucosidase inhibition assay of hexane, ethyl acetate, and methanol along with the standard acarbose were shown in Fig. 1. The concentration for 50% inhibition of hexane, ethyl acetate, and methanol were found to be 950±0.41, 670±0.19, and 503±0.73 µg/ml, respectively, whereas the standard acarbose has an IC<sub>50</sub> value of about 250±0.49 µg/ml which is very effective as compared to all the other extracts being used. Fig. 1 clearly represents that the methanol extract exhibits 78.27% of α-glucosidase inhibition.

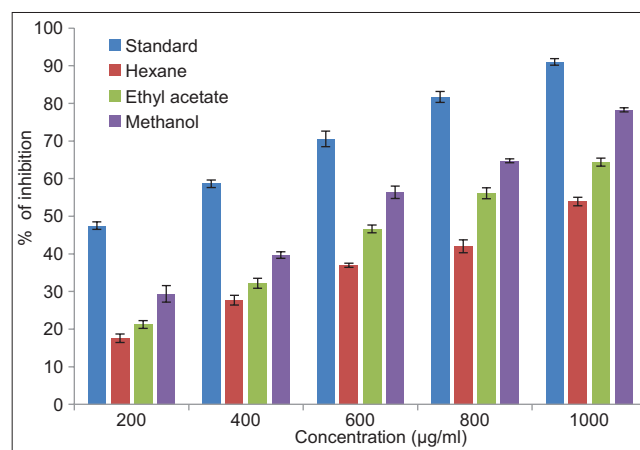
**Statistical analysis**

The data for biochemical and physiological parameters were analyzed and expressed as mean ± standard deviation. The IC<sub>50</sub> values were calculated from linear regression analysis. Results were processed by a computer program, Microsoft Excel (2007).

**DISCUSSION**

The presence of a good number of phytochemicals in the plant extracts screened can be a good source of bioactive components with antimicrobial potency as they can be responsible together with the unscreened ones for the antimicrobial activity of the extracts. Plants containing tannins, alkaloids, saponin, flavonoid, and glycoside showed a broad spectrum of antimicrobial activity [35,36].

The phytochemical screening of hexane extract of *A. bracteolata* leaves indicated the presence of coumarins, steroids, terpenoids, flavonoids, glycosides, carbohydrates, and absence of quinones, phenols, cardiac glycosides, tannins, anthraquinone, alkaloids, saponins, proteins. In the same manner, the presence of steroids, glycosides, carbohydrates were reported in ethyl acetate extract and absences of remaining



**Fig. 1: α-glucosidase inhibition effect of different concentrations (200-1000 µg/ml) of *Aristolochia bracteolata* Linn. Hexane, ethyl acetate, methanol extracts, and standard acarbose. Each value represents the mean±standard error mean of triplicate experiments**

Table 3: Antimicrobial activity of leaf extracts of *Aristolochia bracteolata* Linn. using the disc diffusion method

Zone of inhibition										
Microbes	Hexane (mg/disc)			Ethyl acetate (mg/disc)			Methanol (mg/disc)			Streptomycin 10 µg/disc (Standard)
	1.25	2.5	5	1.25	2.5	5	1.25	2.5	5	
Gram-positive										
96	9	10	11	9	10	11	9	10	11	18
106	8	9	10	11	12	13	9	11	12	20
441	0	0	0	20	22	23	20	22	24	25
3615	0	0	0	0	0	0	9	10	11	20
29212	0	0	0	0	0	0	9	10	11	15
MRSA	8	9	10	8	9	10	9	10	11	21
Gram-negative										
109	0	0	0	0	0	0	0	0	0	12
111	11	12	13	11	12	13	10	12	15	16
451	0	0	0	0	0	0	0	0	0	10
840	0	0	0	14	15	16	18	20	22	16
1771	10	0	0	16	18	19	16	17	20	20
27853	0	0	0	0	0	0	0	0	0	17

0=No activity, Standard - Streptomycin (10 µg/ml) for bacteria. Gram-positive: *Staphylococcus aureus* (MTCC 96), *Micrococcus luteus* (MTCC 106), *Bacillus subtilis* (MTCC 441), *Staphylococcus epidermidis* (MTCC 3615), *Enterococcus faecalis* (ATCC 29212), and MRSA. Gram-negative: *Klebsiella pneumoniae* (MTCC 109), *Enterobacter aerogenes* (MTCC 111), *Vibrio parahaemolyticus* (MTCC 451), *Yersinia enterocolitica* (MTCC 840), *Proteus vulgaris* (MTCC 1771) and *Pseudomonas aeruginosa* (ATCC 27853). MRSA: Methicillin-resistant *Staphylococcus aureus*

Table 4: Minimum inhibitory concentration of *Aristolochia bracteolata* Linn.

Minimum inhibitory concentration (µg/ml)						
Fungi	Strain no.	Hexane	Ethyl acetate	Methanol	Fluconazole 30 µg/ml	
<i>Trichophyton rubrum</i>	MTCC 296	<500	<250	<250	<25	
<i>Aspergillus niger</i>	MTCC 1344	<500	<500	<250	<100	
<i>Botrytis cinerea</i>	MTCC 2880	<250	<250	<250	<100	
<i>Curvularia lunata</i>	MTCC 5109	<500	<500	<250	<12.5	
<i>Trichophyton mentagrophytes</i>	MTCC 8476	<250	<500	<250	<25	
<i>Aspergillus flavus</i>	MTCC10938	<500	<250	<250	<50	

phytoconstituents were confirmed. The results of preliminary phytochemical analysis of leaf extracts revealed the relative distribution of the secondary metabolites which may be responsible for the potent antibacterial activity (Table 1). The methanol extract indicates the presence of phenols, terpenoids, tannins, flavonoids, glycosides, carbohydrates, and remaining phytoconstituents were absent.

Phenolic compounds are a large and complex group of chemical constituents found in plants [37]. They are plant secondary metabolites, and they have an important role as defense compounds. Phenolics exhibit several properties that are beneficial to humans, and its antioxidant properties are important in determining their role as protecting agents against free radical mediated disease processes. Flavonoids are a phenolic structure containing one carbonyl group complexes with extracellular and soluble proteins and with bacterial cell wall [38], thus exhibit antibacterial activity through these complexes. Both phenolic and flavonoid content was assessed by a spectroscopic method where flavonoid compound was found to be more than that of phenolic content. The overall results were represented in Table 2.

The antimicrobial compounds are the group of chemical compounds which either destroy or suppress the growth and metabolism of a variety of microorganism. The antimicrobial efficacy of *A. bracteolata* was revealed by 12 bacterial pathogens including Gram-positive bacteria (*S. aureus*, *B. subtilis*, *M. luteus*, *E. faecalis*, *S. epidermidis*, MRSA) and Gram-negative bacteria (*K. pneumoniae*, *E. aerogenes*, *V. parahaemolyticus*, *Y. enterocolitica*, *P. vulgaris*). When compared with previous reports our research also showed the significant antibacterial activity of ethyl acetate and hexane extracts. Among the three extracts, methanol extract showed the best antibacterial

activity with a zone of inhibition nearly equal to that of the standard streptomycin. *K. pneumoniae*, *P. aeruginosa*, and *V. parahaemolyticus* show no activity at all. Ethyl acetate and methanol extracts were found to be more effective toward Gram-positive than Gram-negative bacterial cultures. The results are represented in Table 3. The study shows the antibacterial activity of *A. bracteolata* for the treatment of the diseases as claimed by the traditional healers. This may be due to the fact that the plant possesses important secondary metabolites such as phenols, flavonoids, glucosides, terpenoids, sterols, lignin, and saponins. The secondary metabolites present in *A. bracteolata* are known to possess antimicrobial activities. In another study, leaf extracts and aristolochic acid from roots of *A. bracteolata* were found to be good antimicrobial agent [39-41]. The species of *Aristolochia*, such as *Heterophylla* and *Kaempferi*, were also reported as good antimicrobial drugs [42-43].

MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation. MICs are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents [44]. In the present study, methanol extract was found to be more effective (250 µg/ml) against all the pathogenic fungi *A. niger*, *B. cinerea*, *T. rubrum*, *C. lunata*, *A. flavus*, *T. mentagrophytes* than ethyl acetate and hexane extracts which are shown in Table 4. Hence, it can act as a good antifungal agent.

Hyperglycemia is believed to increase the production of free radicals and reactive oxygen species, leading to oxidative tissue damage and diabetic complications such as nephropathy, neuropathy, retinopathy, and memory impairment [45]. Glucosidases are a group of digestive



enzymes which break down the dietary carbohydrates into simple monosaccharides. Glucosidase inhibitors such as acarbose reduce the rate of carbohydrate digestion and delay the carbohydrate absorption from the digestive tract. Agents with  $\alpha$ -glucosidase inhibitory activity have been useful as oral hypoglycemic agents for the control of hyperglycemia in patients with diabetes. There are many natural sources with  $\alpha$ -glucosidase inhibitory activity. These studies suggest that preventing an excessive post-prandial rise of blood glucose level by  $\alpha$ -glucosidase inhibition from natural resources are effective in real life as well. Out of all three extracts, methanol extract with  $IC_{50}$  value  $503 \pm 0.73 \mu\text{g/ml}$  was in close proximity to the standard acarbose  $250 \pm 0.49 \mu\text{g/ml}$ , which is clearly shown in Fig. 1.

## CONCLUSION

From the perusal of above studies, we concluded that the methanol extract of the whole plant of *A. bracteolata* can be a good antibacterial agent against Gram-positive and Gram-negative bacteria, and hence it is proved to have a broad spectrum activity which in turn will be helpful in treating various bacterial infections. It can be used as an antifungal agent and can also act as glucosidase inhibitor as well. The plant showed significant glucose inhibition activity, so further the compound isolation, purification, and characterization which is responsible for the inhibiting activity, has to be done for its usage as an antidiabetic agent. On the other side, the presence of flavonoids and phenols concludes that it exerts multiple biological property including antimicrobial, cytotoxicity, anti-inflammatory, and antitumor activities which were believed to act as a powerful antioxidant protecting the human body from free radicals and reactive oxygen species.

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## REFERENCES

- Alluri N, Majumdar M. Phytochemical analysis and *in vitro* antimicrobial activity of *Calotropis gigantea*, *Lawsonia inermis* and *Trigonella foecum-graecum*. *Int J Pharm Pharm Sci* 2014;6(4):524-7.
- Nostro A, Germanò MP, D'angelo V, Marino A, Cannatelli MA. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. *Lett Appl Microbiol* 2000;30(5):379-84.
- Tiwari P, Kumar H, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. *Int Pharm Sci* 2011;1(1):98-106.
- Zheng W, Wang SY. Antioxidant activity and phenolic compounds in selected herbs. *J Agric Food Chem* 2001;49(11):5165-70.
- Cai Y, Sun M, Corke H. Antioxidant activity of betalains from plants of the amaranthaceae. *J Agric Food Chem* 2003;51(8):2288-94.
- Rojas R, Bustamante B, Bauer J, Fernández I, Albán J, Lock O. Antimicrobial activity of selected Peruvian medicinal plants. *J Ethnopharmacol* 2003;88(2-3):199-204.
- Abdullah E, Ahmad Raus R, Jamal P. Extraction and evaluation of antibacterial activity from selected flowering plants. *Am Med J* 2012;3(1):27-32.
- Benkeblia N. Antimicrobial activity of essential oil extracts of various onions (*Allium cepa*) and garlic (*Allium sativum*). *Lebensm Wiss Technol* 2004;37:263-8.
- Elizabeth KM. Antimicrobial activity of *Terminalia bellerica*. *Indian J Clin Biochem* 2005;20(2):150-3.
- Kelmanson JE, Jäger AK, van Staden J. Zulu medicinal plants with antibacterial activity. *J Ethnopharmacol* 2000;69(3):241-6.
- Srinivasan D, Nathan S, Suresh T, Lakshmana Perumalsamy P. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. *J Ethnopharmacol* 2001;74(3):217-20.
- Seliya AR, Patel NK. Ethno medicinal uses of climbers from Saraswati river region of Patan District, North Gujarat. *Ethno Botanical Leaflets* 2009;13:865-72.
- Roy KH, Kumar S, Sarkar S. Wound healing potential of leaf extracts of *Ficus religiosa* on wistar albino strain rats. *Int J Pharm Tech Res* 2009;1:506-8.
- MacMillan DW. The advent and development of organocatalysis. *Nature* 2008;455(7211):304-8.
- Bensky D, Gamble A, Kaptchuk T, Bensky LL. Chinese Herbal Medicine: Material Medical. Revised Edition. Washington, U. S. A.: Eastland Press; 1993. p. 136.
- Tang W, Eisenbrand G. Chinese Drugs of Plant Origin, Chemistry, Pharmacology and Use in Traditional and Modern Medicine. Berlin: Springer-Verlag; 1992. p. 207-22.
- Mizuno M, Oka T, Yamamoto H, Iinuma M, Murata H. Comparison of *Aristolochia* species with chemical constituents. *Chem Pharm Bull* 1991;39:1310-1.
- Kupchan SM, Dorskotch RW. Tumor inhibitors. I. Aristolochic acid, the active principle of *Aristolochia indica*. *J Med Pharm Chem* 1962;91:657-9.
- Meenatchisundaram S, Parameswari G, Subbraj T, Michael A. Studies on antivenom activity of *Andrographis paniculata* and *Aristolochia indica* plant extracts against *Daboia russelli* venom by *in vivo* and *in vitro* methods. *Indian J Sci Technol* 2009;2:76-9.
- Balachandran P, Wei F, Lin RC, Khan IA, Pasco DS. Structure activity relationships of aristolochic acid analogues: Toxicity in cultured renal epithelial cells. *Kidney Int* 2005;67(5):1797-805.
- Negi PS, Anandharamakrishnan C, Jayaprakasha GK. Antibacterial activity of *Aristolochia bracteata* root extracts. *J Med Food* 2003;6(4):401-3.
- el Dirdiri NI, Barakat SE, Adam SE. The combined toxicity of *Aristolochia bracteata* and *Cadaba rotundifolia* to goats. *Vet Hum Toxicol* 1987;29(2):133-7.
- Harborne JB. Introduction of Ecological Biochemistry. 3<sup>rd</sup> ed. London and New York: Academic Press; 1977.
- Samia HA, Elmalik KH, Khalid HS. Therapeutic effect of *Aristolochia bracteolata* extract against experimental *Trypanosoma evansi* infection. *Int J Trop Med* 2006;1(4):170-2.
- Kalpana Devi B, Kanimozhi S, Suganyadevi P. Phytochemical screening and biological property of *Aristolochia bracteolata*. *J Pharm Res* 2011;4(5):1509-14.
- Chitme HR, Malipatil M, Chandrashekhar VM, Prashant PM. Antiallergic activity of *Aristolochia bracteolata* lank in animal model. *Indian J Exp Biol* 2010;48(1):46-52.
- Kavitha D, Nirmaladevi R. Assessment of *Aristolochia bracteolata* leaf extracts for its biotherapeutic potential. *Afr J Biotechnol* 2007;8(17):4242-4.
- Gupta AK, Tandon N, Sharma M. Quality standards of Indian medicinal plants, medicinal plants unit. Indian Council Med Res (ICMR) 2008;5:84-90.
- Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 1<sup>st</sup> ed. London: Chapman and Hall Ltd.; 1973. p. 49-188.
- Slinkard K, Singleton VL. Total phenol analyses: Automation and comparison with manual methods. *Am J Enol Vitic* 1977;28:49-55.
- Willett WC. Balancing life-style and genomics research for disease prevention. *Science* 2002;296(5568):695-8.
- Latha R, Sarkar T, Sagaya JR, Agastian P. Evaluation of antimicrobial efficiency and alpha glucosidase inhibition of *Rubus Ellipticus* smith leaf extracts and its phytochemical analysis. *Asian J Pharm Clin Res* 2015;8(2):422-6.
- National Committee for Clinical Laboratory Standards. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Conidium-Forming Filamentous Fungi: Approved Standard M38-A. NCCLS, Wayne, PA, USA; 2002.
- Dahlqvist A. Method for assay of intestinal disaccharidases. *Anal Biochem* 1964;7:18-25.
- Khan AS, Hassan M, Ali S. Preliminary phytochemical screening of some plants of ethanobotanical importance from district Gilgit, Northern areas, Pakistan. *Pak J Plant Sci* 2009;15:15-8.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: A review. *Int Pharm Sci* 2011;1(1):98-106.
- Walton NJ, Mayer MJ, Narbad A. Molecules of interest: Vanillin. *Phytochemistry* 2003;63(5):505-15.
- Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev* 1999;12(4):564-82.
- Kavitha D, Nirmaladevi R. Assessment of *Aristolochia bracteolata* leaf extracts for its biotherapeutic potential. *Afr J Biotechnol* 2007;8(17):4242-4.
- Manikander RV, Selvamani P, Latha S. Antibacterial activity of leaf extracts of *Aristolochia bracteata* Retz. *Indian J Pharm Sci* 2006;68(4):509-10.
- Angalaparameswari S, Saleem TS, Alagusundaram M, Ramakanth S, Thiruvengadarajan TS, Gnanaprakash K, et al. Anti-microbial activity of aristolochic acid from root of *Aristolochia bracteolata* Retz. *Int J Biol Sci* 2012;8(4):244-7.

42. Wu TS, Chan YY, Leu YL, Chen ZT. Sesquiterpene esters of aristolochic acid from the root and stem of *Aristolochia heterophylla*. J Nat Prod 1999;62(3):415-8.
43. Wu TS, Leu YL, Chan YY. Constituents from the stem and root of *Aristolochia kaempferi*. Biol Pharm Bull 2000;23(10):1216-9.
44. Andrews JM. Determination of minimum inhibitory concentrations. J Antimicrob Chemother 2001;48 Suppl 1:5-16.
45. Maritim AC, Sanders RA, Watkins JB 3<sup>rd</sup>. Diabetes, oxidative stress, and antioxidants: A review. J Biochem Mol Toxicol 2003; 17(1):24-38.