International Journal of Biology Research
www.biologyjournal.in
ISSN: 2455-6548
Received: 05-06-2021, Accepted: 17-06-2021, Published 05-07-2021
Volume 6, Issue 3, 2021, Page No. 05-10

Antibacterial potentials of *Abroma Augusta L.* extractives

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Abstract
Purpose: Antibiotic resistance is a global threat and also one of the major public health challenges at present time. About 2.8 million people have an antibiotic-resistant infection in the U.S. Generally our population used medicinal plants, which drawn attention, that could be an outstanding source of drugs to fight off this problem. This study is paying an attention to exploring the antimicrobial properties of Abroma *Augusta* are used as traditional medicine.
Methods: The chloroform and methanol extracts of the flowers, leaves, root bark, root wood, seeds, stem bark and stem wood of Abroma *Augusta* were tested for their antibacterial potentials against 13 bacteria (6 Gram-positive and 7 Gram negative).Except the flower extract all other extracts offered activity against these pathogenic bacteria.
Result: According to the intensity of activity against the selected bacteria the A. *Augusta* extracts could be arranged in descending order of stem wood>root bark>leaf>root wood>stem bark>seed extract. The minimum inhibitory concentrations (MICs) of the chloroform extract of root wood of A. *Augusta* were 128 µg/ml against S. sonnei, 64 µg/ml against S. dysenteriae and 32 µg/ml against S. - β –haemolyticus.
Conclusion: The present finding showed that the usefulness of different parts of A. *Augusta* plant extracts as natural antimicrobials and suggested the possibility of employing them in drugs for the treatment of infectious diseases.

Keywords: abroma *Augusta*, chloroform and methanol extract, antibacterial activity

Introduction

Antibiotic resistance is a rising problem with 10 urgent threats according to World Health Organization (WHO) listed for 2019.That warns the effective treatment of infectious diseases (Bhatia and Narain, 2010) [22]. More than 35,000 people die every year due to antibiotic resistance. Fighting this threat is a public health Priority, that requires an efficient treatment of a disease to demand the development of new potential sources of novel drugs. Medicinal plants have been accepted as valuable resources of natural antimicrobial compounds as an alternative that can potentially be effective in the treatment of these challenging bacterial infections (Iwu *et al*, 1999. WHO, 1999) [24]. Medicinal plants of our community could be an excellent source of drugs to come to blows off this problem. This study is paying attention to exploring the antimicrobial properties of the plants that are commonly being used as traditional medicines.

A. Augusta, locally known as Ulatkambal, belonging to the family sterculiacae, grows in tropical Asia, South and Eastern Africa, and Australia is found in both wild and cultivated. This plant is used in traditional medicine for medicinal purposes.

The leaves and stems of *A. Augusta* were used by the traditional healers of Bogra district, but the root bark was used by the traditional healers of Jessore district Bangladesh (Sh, *et al*. 2010).

The roots and bark of pivari are used dysmenorrhea, amenorrhea, sterility and other menstrual disorder. The action of dried roots, as well as the sap of the fresh root, has been studied (Kritikar and Basu, 1999) [14].

Different parts of this plant are useful in treating diabetes, stomachache, dermatitis, leucorrhoea, scabies, gonorrhea, cough, leukoderma, jaundice, nerve stimulant, weakness, hypertension, uterine disorders, rheumatic pain and headache with sinusitis (Rahmatullah, *et al.* 2010) [28]. Leaves are useful in treating uterine disorders, diabetes, rheumatic pain and headache with sinusitis (Prajapati, *et al*. 2003) [27], Kumar *et al.* (2011 & 2012) [15]; Sharma *et al*. (2001) [32]; (Dutta and Dutta, 2005) [7]; (Kala, 2000; 2002; 2003; 2004 & 2005) [12, 11, 10]; Shukla *et al*. (2011) [33]. *A. Augusta* is one of the most used plants in Homoeopathic practice in day to day life.

Because of the enormous potentiality of plants as a resource of antimicrobial drugs, this study aimed to explore *in vitro* antibacterial activities of extracts of the different parts of *Abroma augasta* against the most common microbial pathogens.

Materials and Methods

Preparation of plant materials for extraction:

The fresh leaves, flowers, root bark, root wood, stem bark, stem wood and seeds of *A. Augusta* were collected from the campus of the University Rajshahi and different areas of Rajshahi division. All parts of this plant were cut into small pieces and dried at room temperature under shade into the tray which was made of wood. The plant materials were powdered in a grinder separately avoiding excess heat during grinding.

Chemical extraction of the collected materials

Chloroform and methanol were selected to extract seven

different parts of *A. Augusta* separately. The powdered materials were weighed and placed in separate conical flasks to add a sufficient amount of chloroform (500g × 1500ml × 3 times followed by filtration through What man filter paper at 24 h interval in the same collection flask) to yield the first extracts of the seeds of different solvents separately. The output extracts were poured into glass vials and preserved in a refrigerator at 4ºC with proper labeling.

Antibacterial screening: The agar diffusion technique (Bauer *et al*. 1966; Barry *et al*.1980; Vander & Vlietinck, 1991) [5, 4, 35] was employed to conduct antibacterial screening. Standard antibiotic discs of Ciprofloxacin (30µg/disc) were used for comparison. Thirteen pathogenic bacteria(six of which were gram – positive and the rest were gram-negative) were selected for the test and were cultured at the molecular Biology Laboratory, Institute of Biological Sciences, Rajshahi University and Microbiology Laboratory, Rajshahi Medical College, Rajshahi. The test extracts were dissolved in respective solvents in such as manner that the desired concentrations for application in the filter paper disc have been obtained.

Determination of Minimum Inhibitory Concentrations (MIC) for the antibacterial agents: There are two methods for the determination of the MIC:

1. Serial tube dilution technique or turbidimetric assay (Reiner, 1982) and
2. The Paper disc plate technique or agar diffusion assay (Bauer *et. al*. 1966) [5].

Here the serial tube dilution technique was followed using nutrient broth medium to determine the MIC values of chloroform extracts against the following 1 gram positive bacteria, S. *β- haemolyticus* and 2 gram negative bacteria, *S. dysenteriae, S. sonnei.*

MIC was considered as the lowest concentration of the extract that completely inhibits the bacterial growth (*Performance standards for antimicrobial susceptibility testing, 2015*) [20].

Results and Discussion

The antibacterial activities of the test materials were determined by measuring the diameters of the zones of inhibition in terms of mm. The results are shown in tables 1-6. Among the 13 bacteria (6 gram- positive and 9 gram-negative). only seven (*B. cereus, B. megaterium, B. subtilis, S.-β –haemolyticus, S. typhi, S. dysenteriae* and *E. coli*) were responsive to the leaf extract giving promising inhibition zones; *B. cereus, B. megaterium, B. subtilis, S.-β –haemolyticus, S.* typhi, *S. dysenteriae, S. sonnei* and *E. coli* were responsive to root bark extract giving promising inhibition zones; *S. aureus, B. megaterium, S. lutea, S.-β –haemolyticus, S. typhi, S. dysenteriae, S. sonnei, E. coli* and *P. aeruginosa* were responsive to root wood extract giving promising inhibition zones; *B. cereus, B. subtilis, S.-β –haemolyticus, S. dysenteriae, S. sonnei,* and *E. coli* were responsive to stem bark extract giving promising inhibition zones; *B. cereus, B. megaterium, B. subtilis, S.-β –haemolyticus, S. typhi, S. dysenteriae, S. sonnei* and *E. coli* were responsive to stem wood extract giving promising inhibition zones; *S. aureus, B. megaterium, B. subtilis, S. lutea, S.-β –haemolyticus, S. typhi, S. dysenteriae, S. sonnei* and *E. coli* were responsive to the seed extract giving promising inhibition zones respectively at 50 and 200 µg/disc application, al the inhibition zones of the test materials mentioned above were compared to the inhibition zones given by the standard Ciprofloxacin 30 µg/disc and results are mentioned in the Table 1-6.

Table 1: Antibacterial activity of leaf extract of *A. Augusta* and standard Ciprofloxacin

Test organisms	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Ciprofloxacin 30 µg/disc
	50µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria					
S. aureus	-	-		-	30
B. cereus	07	16	09	15	30
B. megaterium	06	14	08	18	30
B. subtilis	07	15			30
S. lutea	-	-	-	-	28
S.-β -haemolyticus	10	22	08	17	30
Gram negative bacteria					
S. typhi	08	18	07	16	30
S. dysenteriae	07	18	06	16	30
S. shiga	-	-	-	-	31
S. sonnei	-	-	--	-	31
S. boydii	-	-	-	-	30
E. coli	08	17	07	15	30
P. aeruginosa	-	-	-	-	30

Table 2: Antibacterial activity of root bark of *A. Augusta and* standard Ciprofloxacin

Test organisms	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Ciprofloxacin 30 µg/disc
	50µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria					
S. aureus	-	-	-	-	30
B. cereus	07	17	06	16	30
B. megaterium	09	20	07	15	30
B. subtilis	08	18	06	14	30
S. lutea	-	-	-	-	31

S.-β -haemolyticus	10	23	07	18	30
Gram negative bacteria					
S. typhi	08	20	07	16	33
S. dysenteriae	07	18	06	14	30
S. shiga	-	-	-	-	30
S. sonnei	07	19	06	15	31
S. boydii	-	-	-	-	31
E. coli	06	17	05	15	30
P. aeruginosa	-	-	-	-	30

Table 3: Antibacterial activity of root wood extract of *A. Augusta* and standard Ciprofloxacin

Test organisms	**Diameter of zone of inhibition (in mm)**				
	Chloroform extract		**Methanol extract**		**Ciprofloxacin 30 µg/disc**
	50µg/disc	**200 µg/disc**	**50 µg/disc**	**200 µg/disc**	
Gram positive bacteria					
S. aureus	07	17	06	14	30
B. cereus	-	-	-	-	30
B. megaterium	09	20	07	16	28
B. subtilis	-	-	-	-	31
S. lutea	08	18	06	15	30
S.-β -haemolyticus	10	22	07	19	30
Gram negative bacteria					
S. typhi	08	18	-	-	33
S. dysenteriae	10	20	07	16	30
S. shiga	-	-	-	-	31
S. sonnei	08	19	06	15	30
S. boydii	-	-	-	-	30
E. coli	07	19	6	16	31
P. aeruginosa	08	15	07	18	30

Table 4: Antibacterial activity of stem bark extract of *A. Augusta* and standard Ciprofloxacin

Test organisms	**Diameter of zone of inhibition (in mm)**				
	Chloroform extract		**Methanol extract**		**Ciprofloxacin 30 µg/disc**
	50µg/disc	**200 µg/disc**	**50 µg/disc**	**200 µg/disc**	
Gram positive bacteria					
S. aureus	-	-	-	-	30
B. cereus	08	18	07	17	30
B. megaterium	-	-	-	-	30
B. subtilis	08	20	05	15	31
S. lutea	-	-	-	-	28
S.-β -haemolyticus	11	21	07	18	30
Gram negative bacteria					
S. typhi	-	-	-	-	33
S. dysenteriae	08	16	07	16	30
S. shiga	-	-	-	-	30
S. sonnei	09	19	07	17	31
S. boydii	-	-	-	-	30
E. coli	07	18	06	14	30
P. aeruginosa	-	-	-	-	30

Table 5: Antibacterial activity of stem wood extract of *A. Augusta* and standard Ciprofloxacin.

Test organisms	**Diameter of zone of inhibition (in mm)**				
	Chloroform extract		**Methanol extract**		**Ciprofloxacin 30 µg/disc**
	50µg/disc	**200 µg/disc**	**50 µg/disc**	**200 µg/disc**	
Gram positive bacteria					
S. aureus	-	-	-	-	30
B. cereus	08	18	07	16	30
B. megaterium	09	22	06	18	30
B. subtilis	08	21	06	14	30
S. lutea	-	-	-	-	28
S.-β -haemolyticus	10	24	08	19	30
Gram negative bacteria					
S. typhi	08	20	06	17	30
S. dysenteriae	09	21	07	18	30
S. shiga	-	-	-	-	30
S. sonnei	07	16	05	15	30

S. boydii	-	-	-	-	30
E. coli	07	15	06	16	30
P. aeruginosa	-	-	-	-	30

Table 6: Antibacterial activity of seed extract of *A. Augusta* and standard Ciprofloxacin

Test organisms	Diameter of zone of inhibition (in mm)				
	Chloroform extract		Methanol extract		Ciprofloxacin 30 µg/disc
	50µg/disc	200 µg/disc	50 µg/disc	200 µg/disc	
Gram positive bacteria					
S. aureus	09	16	06	14	30
B. cereus	-	-	-	-	29
B. megaterium	07	19	05	18	28
B. subtilis	08	22	05	15	30
S. lutea	07	18	06	16	30
S.-β -haemolyticus	11	18	08	17	30
Gram negative bacteria					
S. typhi	09	21	06	16	30
S. dysenteriae	09	20	06	16	30
S. shiga	-	-	-	-	30
S. sonnei	07	18	06	17	28
S. boydii	-	-	-	-	28
E. coli	08	20	05	14	31
P. aeruginosa	-	-	-	-	30

Comparison of the chloroform and methanol extractives

The results showed that increasing concentrations of extracts increased the activities in all the microorganisms. The chloroform extracts of plant parts showed significant antibacterial activity. The stem wood was found to have maximum activity indices against S.-*β –haemolyticus* when tested by the disc diffusion method.

The present results clearly demonstrated that both chloroform and methanol extractives from various parts of *A Augusta* have significant antibacterial properties. The present data on the antibacterial activity of the experimental plant are supported by many recent works. The present findings also fit well with those of Rahmatullah *et al*. (2010b) [28] which found different activities against bacteria. They explained the seed oil of *A. Augusta* Linn has the potential to be an antibacteriall agent against different microorganisms. The oil was screened against various bacteria like *Corynebacterium diphtheria, Escherichia coli, Klebsiella pneumonia, Proteus morganni, Pseudomonas aeruginosa, Salmonella typhi, Shigella boydii, Staphylococcus aureus and Streptococcus pyogenes* for antibacterial activity. These findings also support the findings of Babita gupta *et al*. (2011) [8] which they described *A. Augusta* against different microorganisms.

MIC of *A. Augusta* extracts against the test bacteria:

The extracts found promising during the antibacterial screening have been subjected to the MIC test, especially on the test bacteria the extracts showed activity. The results have been presented in Table 7.

Table 7: Minimum Inhibitory Concentration (MIC) of chloroform extract from (Root wood) against three pathogenic bacteria

Test tube No.	Nutrient broth medium added (ml)	Root wood extract (µg/ml)	Inoculum added (µl)	*S. -β- haemolyticus*	*S. dysenteriae*	*S. sonnei*
1	1	512	10	-	-	-
2	1	256	10	-	-	-
3	1	128	10	-	-	-
4	1	64	10	-	-	+
5	1	32	10	-	+	+
6	1	16	10	+	+	+
7	1	8	10	+	+	+
8	1	4	10	+	+	+
9	1	2	10	+	+	+
10	1	1	10	+	+	+
Cm	1	0	0	-	-	-
Cs	1	512	0	-	-	-
Ci	1	0	10	+	+	+
Results of MIC values in (µg/ml)				32	64	128

"+" = Growth "-" = No growth

The MIC values of the chloroform extract of root wood were 32 µg/ml against *S. - β - haemolyticus*, 64 µg/ml against *S. dysenteriae* and 128µg/ml against *S. sunnei.*

Conclution

Antibiotic resistance is a crisis that continues to challenge the healthcare sector in developing and developed countries. The emergence and spread of multidrug resistant pathogens have substantially threatened the current antibacterial therapy. To, search for a new source of antimicrobial substances such as plants as they produce a variety of bioactive compounds of known therapeutic properties

(Boucher *et al*, 2009, Romero *et al*, 2005, Talbot *et al*, 2006).

. This study has been conducted to assess the antimicrobial activity of *Abroma Augusta* medicinal plant extracts against pathogens

The whole plant has been found to contain several alkaloids and secondary metabolites including steroids, triterpenes, flavonoids, megastigmanes, benzohydrofurans and their glycosides and phenylethanoid glycosides and very effective against a few bacteria and fungi (Gupta, *et al*. 2011) [8]. Phytochemicals like tannin, flavonoids, phenolic compounds, alkaloids, saponins, steroids, cardiac glycosides, phlobatinnins and anthraquinones were also found present/ absent (Duraipandiyan, *et al*, 2006 Djeussi,*et al*, 2013) [17, 18].

Several phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases as urinary tract infections, gastrointestinal disorders, respiratory disease, and cutaneous infections etc.

From the above findings, it is clearly evident that the extracts of different parts of *A. Augusta* are significantly active against most of the bacteria used in this investigation. Thus extensive studies are essential for the isolation of active compound(s) for the development of novel antibacterial agents, especially from the root wood of this promising plant. Further investigations are necessary to evaluate antifungal, antiviral, and antiparasitic activity.

Acknowledgment

The authors are very much thankful to the Director of the Institute of Biological Sciences, University of Rajshahi and also grateful to the director of BCSIR, Rajshahi for providing laboratory facilities.

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