BIOCHEMICAL EVALUTION OF *A.VASICA* FLOWER EXTRACTS FOR THE MANAGEMENT OF BACTERIAL DISEASES

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***Abstract*: Use of antibiotics to treat various diseases is usually a traditional method in use since many years against numerous pathogens in the form of broad spectrum and narrow spectrum antibiotics. But nowadays the major threat posed in the society is the bacterial resistance against many antibiotics so medicinal plants can be used as an alternative means of therapy to combat bacterial infections. Flowers of *A. vasica* were screened for their medicinal property. The antibacterial activity was analysed by preparing its extract in different solvents of increasing polarity like ethyl acetate (S1), methanol (S2) and aqueous (S3). Gentamicin was used as control against Gram positive and Gram negative bacteria (*S. aureus, B. subtilis, Pseudomonas aeruginosa, Salmonella typhi, E. coli*) known to cause Food borne infections. Different extracts of flowers of *A.vasica* exhibited a varied degree of inhibition against selected human pathogens. The highest degree of inhibition was observed (ZOI) in ethyl acetate (S1) as 14mm, methanol (S2) extract as 14mm and aqueous (S3) extract as 17mm. The phytochemical analysis was also done to support the data generated by antimicrobial activity. Results of phytochemical analysis showed the presence of Phenolics, Saponins, Flavonoids, Ascorbic acid, Tannins and Alkaloids**

***Keywords*: *A. vasica*, ZOI, Phenolics, Saponins, Bacterial resistance.**

INTRODUCTION

Our mother nature has blessed us with enormous variety of flora, and the plants from prehistoric time are well known for their medicinal property and various parts of plants are used for the preparation of medicines for various ailments and also to cure various food born infections caused by various pathogens. Plants make many chemical compounds to protect themselves against fungi, bacteria or mammals and this act as same way on human body as allopaethic drugs. Now, these properties of plants can be used to replace the antibiotics, to which various bacteria are showing resistance because of the frequent use of drugs. New drugs with better effects are needed to provide good health facility. Another benefit of these drugs is that they are safe and cost-effective which is born for the non-industrial and under developed countries. Another reason for the development of these drugs is population rise, prohibitive cost of treatment, side effects of other synthetic drugs. India is a large repository for herbal plants in world. About 8000 herbals are codified in AYUSH systems in India. Worldwide, 80 percent of people rely on herbs, notified by WHO (World Health Organization). As per data three-quarter of population rely on plants and their extract for health care **(https://en.m.wikipedia.org>wiki>medicinal plants)**. Food borne infections are the major type of infections which are prevailing in the society due to the increased use of adulterated food and junk foods, where the hygienicity of food in compromised at certain level and the infections spreading due to the usage of contaminated water are also very common, which is caused by the bacteria *E.Coli* and some other food borne infections are typhoid due to *S.typhi*, diarrohea caused by spps. of *Bacillus*, several food products spoilages are due to spps. of *Pseudomonas* and several gastrointestinal illness caused due to *Staphylococcal* spps **(https://nhp.gov.in)**. One of the herbal plants known as *A.vasica* which are known to have several chemicals such as alkaloids, glycosides, polyphenols, terpenes etc. which have the power to combat the food-borne illness. The plant *A.vasica* is commonly known as Malabar nut come from Acanthaceae family native to Asia. The plant ranges in Sri Lanka, India, Bangladesh, Pakistan, Indonesia and Malaysia **(Kanthale et al.,2014)**. All parts of the plants are full of medicinal property (leaves, barks, roots and flower). If we focus on food borne infections than *A.vasica* have the several properties to fight several infections regarding food. The *A.vasica* flower are screened for their phytochemical content. Quantitative test were used to detect the presence of alkaloids, tannins, flavonoids, saponins, phenolic acid. Presence of these phytochemicals in the medicinal plants indicates the presence of antibacterial properties against *S.aureus, E.coli, Psuedomonas aeruginosa, Bacillus subtilis* and *S.typhi.* The study was carried out to screen the bioprotective properties of *A.vasica* flowers for its use as an effective antimicrobial agent to cure various infections caused by human pathogens. To analyze the phytochemicals responsible for significant antimicrobial activity, phytochemical analysis was also performed.

MATERIAL AND METHODS

Plant material and extract

The mature flowers of *Adhatoda vasica* were collected from local area in Bhaniyawala Dehradun, India. Washed with distilled water and flower were separated and kept in a clean shaded place for 9-10 days, grounded to a powder and weighed the whole powder. Cold extraction method is used to prepare S1 and S2 extract and S3 extract is prepared by decoction i.e. vigorous boiling of 25gm powder for 2-3 hours.**(Akhter et al., 2014, Bele, A., et al 2009)**.

Chemicals and Reagents

Chemicals and reagents used are for the study are ethyl acetate, methanol, Molisch's reagent, sulphuric acid, ferric chloride, NaOH, dragondroff's reagent and distilled water. These reagent and chemical are used in a pure state.

Microorganisms

Five microorganisms representing gram positive and gram negative bacteria were used. Which are *E.coli* (P1), *B.subtilis* (P2), *P.aeruginosa* (P3), *S.typhi* (P4) and *S.aureus* (P5).

Antimicrobial activity

Agar well diffusion method

Antibacterial activities of all the extracts (S1, S2, and S3) of *A.vasica* were determined by agar well diffusion method. In this method DMSO (dimethyl sulphoxide) was dissolved in the extract to obtain 0.5gm/100µl and 1gm/100µl concentrations. Commercial antibiotic (Gentamicin) and DMSO was taken as positive and negative control respectively. **(Sawhney et al., 2011).**

Broth dilution MIC test

The Minimal Inhibitory Concentration (MIC) of the plant extracts was determined by macro broth dilution assay (20). On the basis of the results obtained from Agar well diffusion method (ZOI) two-fold serial dilutions of all the extracts were prepared in well plates with Mueller-Hinton Broth (Hi-media, Mumbai, India) as diluents. 20µl of test microorganisms of the standard concentration (5 × 105 cfu/ml) was inoculated in the each dilution. Two-fold serial dilution of DMSO and gentamicin was used as experimental negative and positive control respectively. The plates were incubated at 37^0C for 24hours. The lowest concentration at which the extract or standard drug showed no visible growth (turbidity) was taken as the MIC.

Determination of Minimum Bactericidal Concentration

20µl of the MIC test broth tube solutions were spread over MHA plates and incubated for 18-24h at 37^0C. The plates showing no single bacterial growth, the dilution was considered as MBC (Minimum Bactericidal Count) concentration of the extract that is bactericidal in nature. The MIC index (MIC/MBC) was performed to determine whether an extract is bactericidal (MIC/MBC <4) or bacteriostatic (MIC/MBC >4) in nature. MIC index values of greater than 4 and less than 32 are considered as bacteriostatic (21). The test was performed in triplicates and its mean MIC and MBC values were calculated. The results were expressed in terms of standard deviation.

Phytochemicals analysis

Phytochemicals analysis was done in accordance to **(Sharma et al., 2014)**.

Test for glycosides

Take 1ml of plant extract and add few drops of sulphuric acids and the mixture was allowed to stand for some time, formation of Reddish precipitate that means presence of glycosides was confirmed.

Test of carbohydrates (Molisch's test)

Take 1ml of extract and add 2ml of Molisch's reagent now to this mixture, 2ml conc. Sulphuric acid was added along the sides of the test tube. Presence of carbohydrates was confirmed by formation of reddish violet ring.

Test of flavoniods (Aqueous test)

Take 1ml of plant extract, 1ml of aqueous NaOH was added. Presence of flavonoids was confirmed by yellow colour formation.

Test for saponins (Aqueous test)

Take 1ml of extract, 5ml water was added and shake well in test tube shaker. Presence of saponins was confirmed by Lather formation.

Test for tannins (Ferric chloride test)

Take 1ml of plant extract, 1ml of ferric chloride was added. Presence of tannins was confirmed by the formation of greenish black colour.

Test for alkaloids (Dragondroff's reagent)

Take 1ml of plant extract add 5-6 drops of dragondroff's reagent. Presence of alkaloids was confirmed by the formation of creamish/brownish-red/orange precipitate.

RESULTS AND DISCUSSION

Antibacterial activity

Various extract of *A.vasica* flower showed potent antibacterial activity against the pathogens causing infections in human. S1 and S3 extracts were found to be most potent in inhibiting the growth of selected pathogens as the values of ZOI obtained were significant. In S1 extract, maximum inhibition was observed against *Psuedomonas* at 1mg/100µl (ZOI 14mm) whereas the least inhibition was against *S.typhi* with ZOI (11mm at 1mg/100ul). In the S2 extract, *Pseudomonas* was inhibited effectively at the 1mg/100ul (17mm) followed by *S.typhi* (12mm at 1mg/100µl), *B. subtilis* (10mm at 1mg/100µl), *S. aureus* (10mm at 1mg/100µl) and *E.coli* (9mm at 1mg/100µl). In the S3 extract the higher ZOI was obtained against *Psuedomonas* with ZOI (17mm at 1mg/100ul) followed by *E.coli* (13mm at 1mg/100ul). The data obtained from antimicrobial activity of all the 3 extracts against selected pathogens were tabulated in table 1 and represented graphically as Fig: 1,2,3,4. Out of all the selected Gram positive and Gram negative pathogens for the antimicrobial study, *Pseudomonas aeruginosa* was found to be the most susceptible pathogen by different solvent extracts of the *A. vasica* flower extracts. The extracts that showed high efficacy against pathogens was subjected to minimum inhibitory concentration (MIC) assay by two-fold serial dilution method (table 2,3 and 4 MIC table).

Table: 1 Represents the antimicrobial activity of S1, S2, S3 extracts and Gentamicin (Positive Control) as ZOI in mm against human pathogens.

Microbial Culture	Concentration							
	0.5mg/100µl				1mg/100µl			
	Gentamicin	S1	S2	S3	Gentamicin	S1	S2	S3
P1	31mm	10mm	8 mm	14mm	35mm	12mm	9mm	13mm
P2	26mm	8mm	9 mm	12 mm	30mm	13mm	10 mm	14 mm
P3 pseudomonas	27mm	7mm	13mm	17 mm	32mm	14mm	14 mm	17 mm
P4	21mm	8mm	9 mm	12 mm	28mm	11mm	12 mm	15 mm
P5	22mm	9mm	8mm	13 mm	27mm	12mm	10mm	14 mm

Fig: 1 Represents the graphical antibacterial activity of Gentamicin of *A.vasica* against bacterial culture.

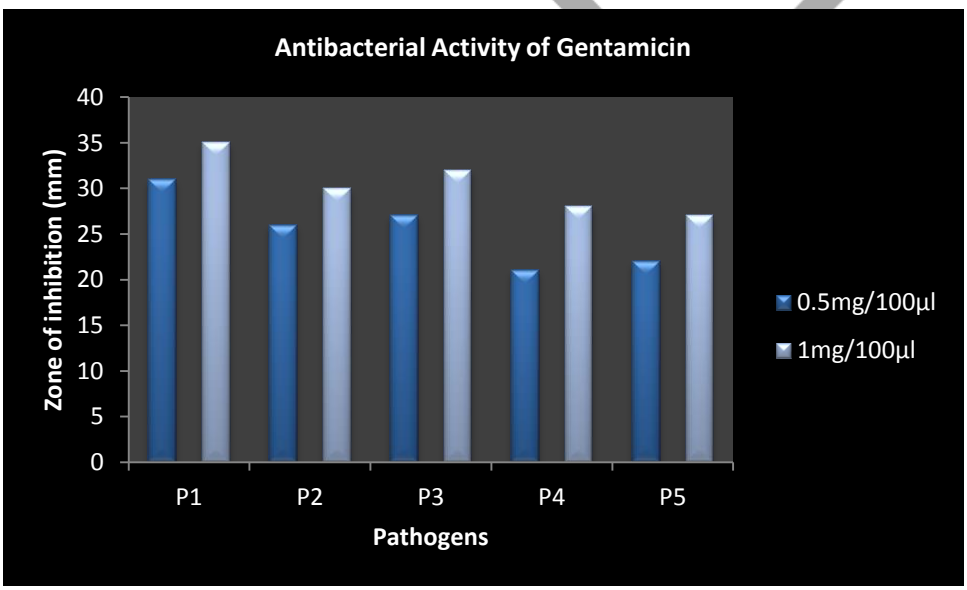


Fig: 2 Represents the graphical antibacterial activity of S1 extract of *A.vasica* against bacterial culture.

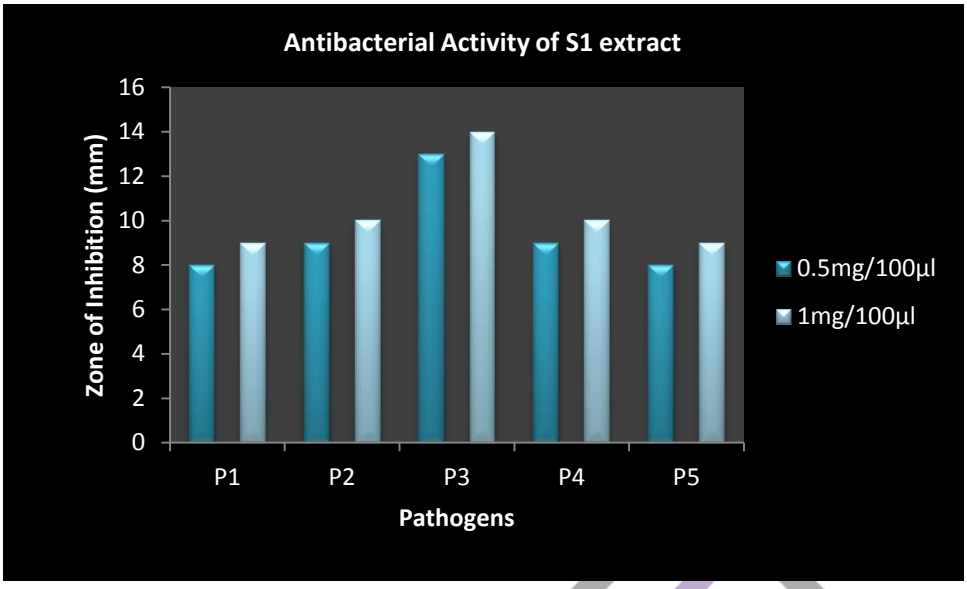


Fig: 3 Represents the graphical antibacterial activity of S2 extract of *A.vasica* against bacterial culture.

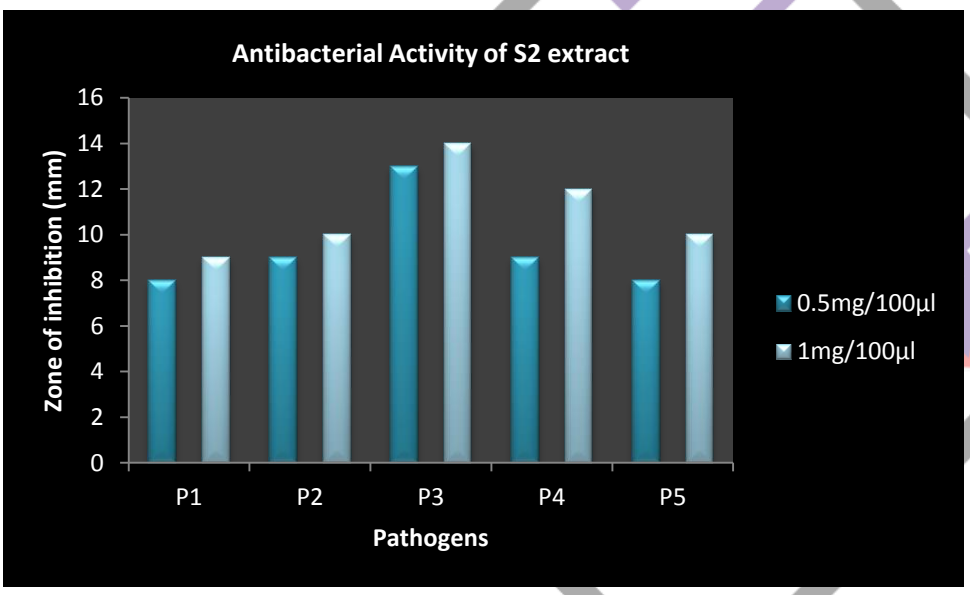
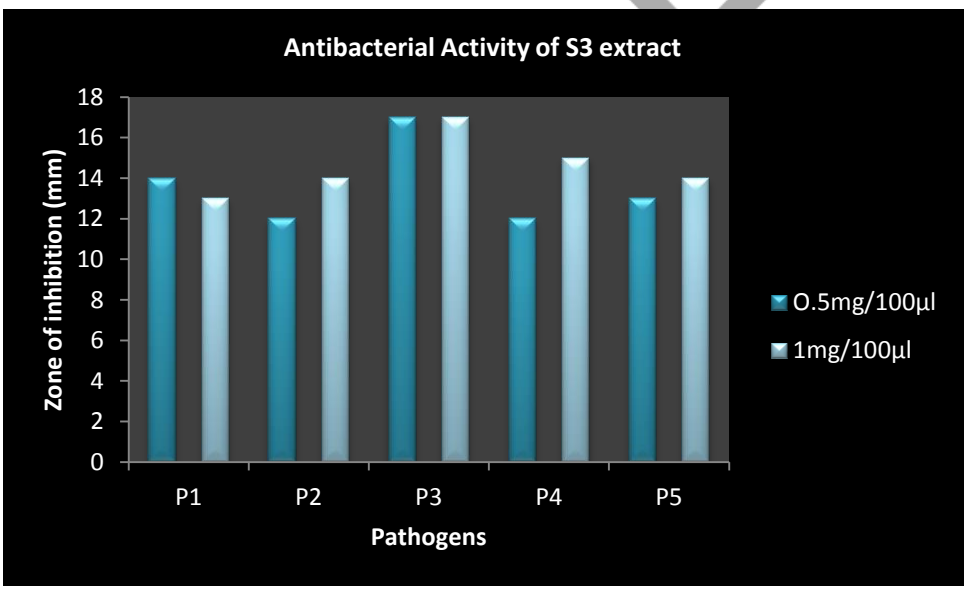


Fig: 4 Represents the graphical antibacterial activity of S3 extract of *A.vasica* against bacterial culture.



MIC (Minimum Inhibitory Concentration)

MIC is the minimum concentration of the extract which is required to inhibit the bacterial population. The extract which showed highest inhibition of microorganisms were subjected to minimum inhibitory concentration (MIC) assay by two-fold serial dilution method (**Florey et al., 1989 and Drummond et al., 2000)**. The S1 extract was found to be most effective in inhibiting P5 i.e. 0.0625mg/ml followed by P1, P2, P3 and P4 at 125mg/ml. P1 was the most susceptible pathogen against S2 extract (0.125mg/ml) and all the pathogens were inhibited at the value of 0.25mg/ml for all the pathogens. The S3 extract inhibited all the selected pathogens at 0.125mg/ml. Out of the three extracts the significant MBC values were exhibited by S1 extract at concentration of 125mg/ml and 0.25mg/ml. **Elgal et al., 2017** in their study done on *A.vasica* extract of the flower, showed that in S1 extract both *E.coli* and *S.aureus* were inhibited by MIC concentration of 3.125mg/ml followed by *Psuedomonas* (MIC 12.5mg/ml) whereas in our study the effective MIC concentration was recorded against *S. aureus* i.e 0.0625mg/ml of S1 extract.

Table 2: The MICMBC and MIC Index value of ethyl acetate (S1) extract against different pathogens.

Pathogen	Range (mg/ml)	MIC (control mg/ml)	MBC (control mg/ml)	MIC (extract) (mg/ml)	MBC (extract) (mg/ml)	MIC Index (control)	MBC Index (extract)
P1	0.5-0.0156	0.0156	0.0312	0.125	0.25	2	2
P2	0.5-0.0156	0.0156	0.0312	0.125	0.25	2	2
P3	0.5-0.0156	0.0156	0.0312	0.125	0.25	2	2
P4	0.5-0.0156	0.0156	0.0312	0.125	0.25	2	2
P5	0.5-0.0156	0.0156	0.0312	0.0625	0.125	2	2

Table 3: The MIC, MBC and MIC Index value of methanol (S2) extract against different pathogens.

Pathogen	Range (mg/ml)	MIC (control) (mg/ml)	MBC (control (mg/ml)	MIC (extract) (mg/ml)	MBC (extract) (mg/ml)	MIC Index (control)	MIC Index (extract)
P1	0.5-0.0156	0.0156	0.0312	0.125	0.25	2	2
P2	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P3	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P4	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P5	0.5-0.0156	0.0156	0.0312	0.25	0.25	2	2

Table 4: The MIC,MBC and MIC Index value of aqueous (S3) extract against different pathogens.

Pathogen	Range (mg/ml)	MIC (control) (mg/ml)	MBC (control) (mg/ml)	MIC (extract) (mg/ml)	MBC (extract) (mg/ml)	MIC Index (control)	MIC Index (extract)
P1	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P2	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P3	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P4	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2
P5	0.5-0.0156	0.0156	0.0312	0.25	0.5	2	2

Phytochemical analysis of *Adhatodha vasica* flower extracts

Qualitative analysis

Results obtained from phytochemical analysis of *A.vasica* flower extract showed the presence of varied significant botanicals that exhibited potent antimicrobial activity. Qualitative Phytochemicals analysis of *A.vasica* flower (S1,S2, S3) extract exhibit the presence of Saponins, carbohydrates, flavonoids, tannins and alkaloids. Glycosides are absent in all the extracts. Results are presented in table no.5. Phytochemicals such as Saponins, Flavonoids, Tannins and Alkaloids provide strongprotective activity in terms of antibacterial properties to the medicinal plant against *S.aureus, S.typhi, E.coli, Bacillus* and *Pseudomonas.* Saponins are the chemical compounds found in wide range of plants and several studies confirm the health benefits of saponins. These chemicals may help in reducing cholesterol level, inhibit disease causing bacteria and inhibit tumor growth.

Table 5: **Represents the Phytochemical analysis of *A.vasica* flower extracts.**

S.NO.	Phytochemicals	Ethyl acetate extract	Methanolic extract	Aqueous extract
1	Glycosides	-ve	-ve	-ve
2	Carbohydrates	+ve	+ve	+ve
3	Flavonoids	+ve	+ve	+ve
4	Saponins	+ve	+ve	+ve
5	Tannins	+ve	+ve	+ve
6	Alkaloids	+ve	+ve	+ve

The S1, S2 and S3 extracts obtained from the flower of *A.vasica* were found to be strongly active against the selected human microbes. Effect: 1. They inhibit cell synthesis 2. They stop microbial protein and nucleic acid synthesis 3. They disrupt microbial membrane structure and function 4. They block metabolic pathway through inhibition of key enzyme. **(Sawhney et al., 2011)**. These pathogens cause various types of diseases in human. In all the 3 extract- ethyl acetate, methanol and aqueous extracts the presence of alkaloids, flavonoids, terpenoids, tannins and glycosides was detected. In the study conducted by **Ramachandran et al., 2013** the presence of phytochemicals was also seen. In our study we have focused on searching the novel drug therapy in the form of plant based formulations for food-borne infections caused by gram positive and gram negative bacteria in humans. The phytochemicals present in *A.vasica* posses the potentiality to cure various bacterial afflictions and have negligible or no side effects. Various of phytochemicals such as alkaloids, Tannins, saponins, flavonoids etc., have exhibited effective range of antimicrobial activity against gram negative as well as gram positive pathogens so it can be used as an active ingredient in the plant based formulations as a cure for different ailments.

Conclusion:

Medicinal plants possess significant botanicals with numerous pharmacological activities with high safety profile and negligible side effects. From our study it can be concluded that *A.vasica* fruit extracts were found to be strong inhibitory agents and also loaded with active ingredients which can be used in the preparation of herbal formulations to combat various diseases caused by gram negative and gram positive human pathogens. The study is extendable to in vivo experiments for the determination of specific mode of action of extract. So Plant based therapeutics needs to be encouraged as an alternative means of therapy in the era of drug resistance.

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