International Journal of Bio-pharmacology, Biotechnology and Allied Sciences

IJBBAS, April, 2020, 1(1): 29-38 Research Article www.ijbbas.in

PHYTOCHEMICAL AND ANTIBACTERIAL ASSAY OF METHANOLIC LEAVES EXTRACT OF *C. ROSEUS*, S. *INDICA, P. GUAJAVA* AND *B. PINNATUM* AGAINST *S. AUREUS* AND *S. ENTERIDI*

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Available online at: www.ijbbas.in.

Received 20th Jan. 2020; Revised 26th Feb. 2020; Accepted 10th march. 2020; Available online 17th April.

ABSTRACT

Plants are the best source for the formation of herbal drugs by showing various antibacterial activities against bacterial pathogens. Some of these plants are more operative than common antibiotics to combat the pathogenic microorganisms studied. According to the *invitro* screening it was found that the *S. indica* commonly known as ashoka shows effective and best results 34.1 mm and 16.5 mm against *Staphyllococcus aureus* and *Salmonella enteridi* The phytochemical analysis of methanolic leaves extract of *Catharanthus roseus, Psidium guajava, Bryophyllum pinnatum and Saraca indica* was also performed and concluded that the activities observed could be due to the presence of phytochemicals like, Tannins, saponins, terpenoids, flavonoids,etc. which were detected.

Key words: Antibacterial, ZOI, Photochemical, *in vitro*

INTRODUCTION

Plants, animals or microorganism are the natural sources from where the drugs can be obtained. The plant kingdom is the storehouse of the organic compounds **[1].** Plant drug Rasayana has always played a vigorous role to treat several diseases of human beings. According to World health organization (WHO) more than 80% of the world population depends on traditional medicine for their health care needs **[2].** Diseases like, tuberculosis, bubonic plague, the human immune deficiency, virus acquired immunodeficiency syndrome pandemic etc. affected the large portions of the human population, causing significant morbidity and mortality. For curing such diseases numerous medicinal plants are in use **[3].**

Throughout the world, there are 250,000-500,000 plant species, out of which only few species have been scrutinized phytochemically and their extracts are studied biologically or pharmacologically screening. The compounds either extracted from natural source of designed synthetically have been the source of countless therapeutic agents **[4] [5] [6].** Medicinal plants are rich sources of antimicrobial agents. Plants are used medicinally in different countries and are the source of potential and powerful drugs **[7].**

Although many plant species have been tested for antimicrobial properties, the majority of these have not been adequately evaluated **[8].** In present time there are lots antibiotics are in daily use, but due to resistance problem demands that a renewed effort be made to screen various medicinal plants for their potential antimicrobial traits, which are due to compounds synthesized in the secondary metabolites of the plant. Alkaloids, flavonoids, tannins,phenolic compounds, steroids, resins, fatty acids and saponins are some secondary metabolites of plants which are capable of producing definite physiological action on body **[9].**These naturally occurring compounds are also able to show defense mechanism and protect the particular from various diseases **[10].**

Many of the whole plants or plant materials are used in traditional medicine is generally proved more effective and relatively cheaper than modern medicine **[11]** against certain illnesses while concurrently justifying various side effects that are often associated with synthesized drugs or antimicrobials **[12].**

Phytocompounds are known for their unique properties and activities such as terpenoids which shows various important pharmacological activities i.e.,

anticancer, anti-inflammatory, anti-malarial, inhibition of cholesterol synthesis, anti-bacterial, anti-viral activities **[13],** alkaloids are used as Anesthetic agents and are found in medicinal plants **[14].** The main objective of the work is checking the antibacterial activity against resistive pathogens and identification of the phytocompounds in respective plants.

Material Methods

Extraction of active metabolites

The four types of leave samples were collected from four different areas of the Bareilly region, (Uttar Pradesh), such as *Catharanthus roseus (sdabahar)*, *Bryophyllum pinnatum* (Ajooba), *Psidium guajava (guava)*, *Saraca indica (ashoka).* The dried leaves powder were dipped into methanol (1:10 w/v ratio) and incubated for 48 hours. The filtrate was dried, to obtain the desired metabolite. Then metabolite was resuspended in dimethyl sulphoxide (DMSO) and preserved at -20°C for screening the antibacterial properties **[15].**

Tested microorganisms

Bacterial pathogens, *S. aureus* and *S. enteridi,* sub-cultured and maintained at Invertis University, Bareilly, Uttar Pradesh.

Antibacterial screening

The screening was done by agar well diffusion method reported by Akiyana, H et al., 2011 **[16].** The 24 hours grown bacterial pathogens were spread on sterilized nutrient agar plates and then four wells were created by using sterile borer (5mm). The plant extracts of 1 mg/ml concentration, DMSO as negative and tetracycline as positive control was loaded to wells. The activity was screened on the basis of zone of inhibition.

Minimum Inhibitory Concentration Test

The test was executed by broth dilution method as described by Rodriguez-Tudela JL et al., 2003 **[17].** The samples were serially diluted in broth with pathogens and then incubated at 37° for 24 hours. The MIC value for obtained, after taking optical density at 620 nm by sing spectrophotometer.

Phytochemical Analysis

Phytochemical analysis or screening is the type of chemical assay which is used for the identification of the presence of various phytochemicals such as alkaloids, flavonoids

steroids etc. in the plants **[18].**

Test for Alkaloids: 2% H_2SO_4 was added to 1 ml of plant extracts with 1mg/ml concentration and warmed for 2-5 minutes. Few drops of Dragendrofs reagent were added to filtered sample and observed for the presence of orange red precipitates for the presence of alkaloids.

Test for Terpenoids and Sterols: 1 ml of plant extract (1mg/ml) was added to 2 ml of $CHCl_3$ and 2ml concentrated H_2SO_4 carefully to form a layer. Then the presence of reddish brown colour interface, show positive results for the presence of terpenoids and sterols.

Test for reducing sugars: 2 ml Benedict reagent was added to 5 ml plant extract of 1mg/ml concentration and then boiled for 10-15 minutes. The orange red precipitate indicates the presence of reducing sugars.

Test for Saponins: 5 ml of distilled was added to 1 ml of each plant extract (1mg/ml) and shaken vigorously. The frothing (appearance of creamy mass of small bubbles) was observed, this shows the presence of Saponins.

Test for Tannins and Phenols:1 ml plant extract of 1mg/ml concentration was taken and mixed with 2ml distilled water, then heated at 60°C for 5 min. Then the mixture was filtered and 2% $FeCl_3$ was added to the filtrate. Dark green solution indicates the presence of tannins.

Test for Flavonoids:1 ml diluted NaOH and 1ml diluted HCl was added to the 1 ml plant extract with 1mg/ml concentration and observed for yellow solutions that turns colourless, which indicate the presence of flavonoids.

Test for Phlobatannins: 1 ml plant extract (1mg/ml) was mixed in 1ml distilled water and then filtered. The filtrate was boiled with 2ml of 2% HCl solution.The red precipitate shows the presence of Phlobatannins.

RESULTS

Sample collection and extract preparation

The leaves of *Catharanthus roseus* (Sdabahar),*Bryophyllum pinnatum* (Ajooba), *Psidium guajava* (Guava), *Saraca indica* (Ashoka) were collected, washed and then dried. The dried leaves were grinded and converted into powder. After that the powder was dipped in methanol (1:10 w/v ratio) and after 48 hours filtered in h weighted bowl, then after evaporation of solvent the residue was resuspended in DMSO and screened for antibacterial properties.

a: *Saraca indica* b: *Catharanthus roseus*

c: *Bryophyllum pinnatum* d: *Psidium guajava*

Figure 1: *Leaves sample of respective plants a: Saraca indica (Ashoka), b: Catharanthus roseus (Sdabahar)*, c: *Bryophyllum pinnatum* (Ajooba), d: *Psidium guajava (Guava)*.

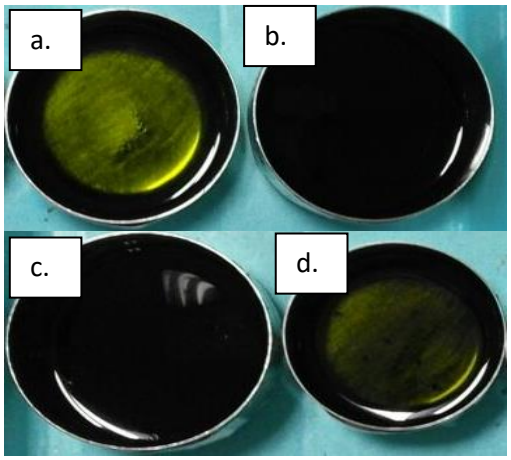

Figure 2: Collected methanolic extracts in weighted bowl of plants *a: Saraca indica (Ashoka), b: Catharanthus roseus (Sdabahar)*, c: *Bryophyllum pinnatum* (Ajooba), d: *Psidium guajava (guava)*.

Antibacterial Screening

1 mg/ml concentration of extract were loaded to well and compared with positive and negative controls. After observing the results it was found that methanolic extractof *S. indica* shows maximum antibacterial property as compare to tetracycline (positive control) as shown in table 1 and figure 3.

Table 1: Antibacterial activity of methanolic extracts of *Saraca indica (Ashoka), Catharanthus roseus (Sdabahar)*, *Bryophyllum pinnatum* (Ajooba), and *Psidium guajava (Guava)* against *S. aureus* and *S. enteridi.*

Pathogen	**Zone of Inhibition (mm)**	
	S. aureus	*S. enteridi*
C. roseus	11	15
B. pinnatum	11.5	11.5
P. Guajava	0	14
S. indica	34.1	16.5
Tetracycline	23.5	13.5

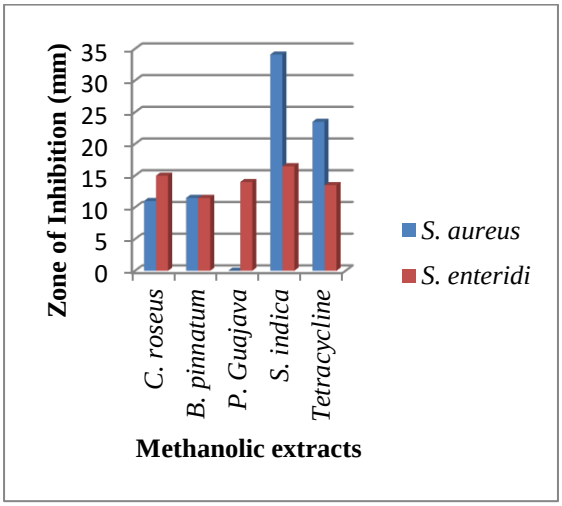

Figure 3: Antibacterial activity of methanolic extracts of *Saraca indica (Ashoka), Catharanthus roseus (Sdabahar)*, *Bryophyllum pinnatum* (Ajooba), and *Psidium guajava (Guava)*.

Minimum Inhibitory Concentration Test

The extracts with concentration 1 mg/ml were diluted and in presence of pathogens and after 24 hours of incubation at 37°C the optical density was checked. Where it was found that the extract of *S. indica found very effective MIC value of* 0.125 mg/ml; where as *C. roseus*, *P. guajava* indicates 0.5 mg/ml and *B. pinnatum* represent 0.25 mg/ml against *S. aureus.* In the presence of *S. enteridi, S. indica shows* 0.25 mg/ml, *C. roseus*, *B. pinnatum* and *P. guajava indicates* 0.5 mg/ml value with respect to tetracycline as mentioned on table 2 and figure 3.

Table 2: MIC values of methanolic extracts of plants against bacterial pathogens.

Sample	*S. aureus*	*S. enteridi*
S. indica	0.125 mg/ml	0.25 mg/ml
C. roseus	0.5 mg/ml	0.5 mg/ml
B. pinnatum	0.25 mg/ml	0.5 mg/ml
P. guajava	0.5 mg/ml	0.5 mg/ml
Tetracycline	0.25 mg/ml	1 mg/ml

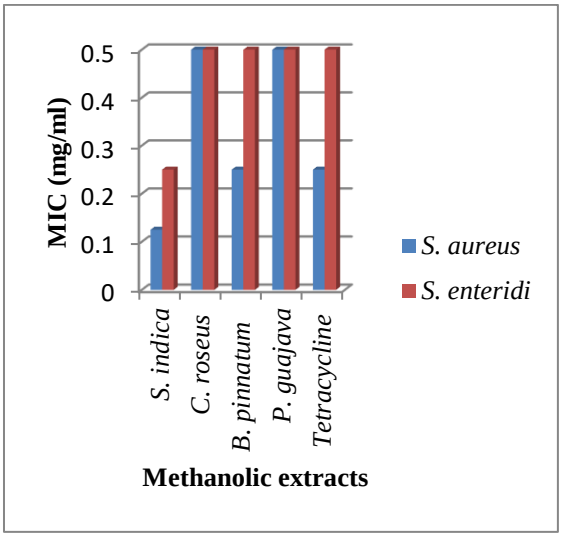

Figure 4: MIC values of methanolic extracts of plants with respective to positive control against bacterial pathogens.

Phytochemical Analysis

The extracts were screened for checking the presence of phytochemicals with colour indication and the results defined in Table 3.

Table 3: Phytochemical analysis of the methanolic-leaves extracts of plants

***C. roseus*:**

Phytochemicals	**Colour Indication**	**Result**
Alkaloid test	Orange red precipitates	Positive
Terpanoid and Sterol test	Reddish brown colour	Positive
Reducing sugar test	Greenish colour	Negative
Saponins test	Frothing occurs	Positive
Tannins and phenols test	Dark green colour	Positive
Flavonoids test	Colourless	Positive
Phlobatannins test	Green colour	Negative

B. pinnatum

Phytochemicals	**Colour Indication**	**Result**
Alkaloid test	Orange red precipitates	Positive
Terpanoid and Sterol test	Reddish brown colour	Positive
Reducing sugar test	Orange red precipitates	Positive
Saponins test	No frothing	Negative
Tannins and Phenols test	Dark green colour	Positive
Flavonoids test	Colourless	Positive
Phlobatannins test	Pale yellow colour	Negative

P.guajava

Phytochemicals	**Colour Indication**	**Result**
Alkaloid test	Orange red precipitates	Positive
Terpanoid and Sterol test	Reddish brown colour	Positive
Reducing sugar test	Orange red precipitates	Positive
Saponins test	No Frothing	Negative
Tannins and Phenols test	Dark Green colour	Positive
Flavonoids test	Colourless	Positive
Phlobatannins test	Green colour	Negative

S. indica

Phytochemicals	Colour Indication	Result
Alkaloid test	Orange red precipitates	Positive
Terpenoid and Sterol test	Reddish brown colour	Positive
Reducing sugar test	Orange red precipitates	Positive
Saponins test	No frothing	Negative
Tannins and Phenols test	Dark green colour	Positive
Flavonoids test	Yellow colour	Negative
Phlobatannins test	Yellowish colour	Negative

DISCUSSION

For curing various diseases and minimizing the side effects of synthetic drugs, pharmaceutical industries showing their interest towards the phytochemicals and antibacterial compounds of the plants for the formation of the new drug. It is expected that the important phytochemical properties recognized by our study in the indigenous plants will be very useful in the curing of various diseases. From the results it was found that the *S indica* shows great antibacterial properties as compared to the known drug tetracycline by showing zone of inhibition 34.1 mm against *S. aureus* and 16.5 mm against *S. enteridi.* The MIC value was also calculated, which indicates 0.125 mg/ml against *S. aureus* and 0.25mg/ml against *S. enteridi.* The phenolic compounds of plants such as tannins, flavonoids, and phenolic acids seemed to be strong antioxidant and antiradical compounds. The presence of a 2,3-double bond, orthodiphenolic structure and the number of hydroxy groups enhance the antioxidative activity of flavonoids. The glycosylation, blocking the 3-OH group in C-ring, lack of a hydroxy group or the presence of only a methoxy group in B-ring have a decreasing effect on antiradical or antioxidative activity of these compounds **[19,20].**Alkaloids, as the name derived from the word alkaline which is used to define any nitrogen-containing base or organic compound. Alkaloid shows various pharmacological effects and used as recreational drugs, as medications, or in entheogenic rituals **[20].**

Similarly the secondary metabolites of the plants help the f or showing resistant against the various pathogens either bacteria, fungus, protozoa etc. they directly or indirectly inhibit the growth / multiplication of the pathogens. By performing the phytochemical analysis is was observed that the plant containing the secondary metabolite or the chemicals such as flavonoids, phenols, alkaloids, reducing sugars etc. plays a vital role in inhibiting the growth of pathogen. And the PE shows their resistant power against the culture by makingthe ZOI in the culture plate during the antimicrobial assay.

CONCLUSION

All the plant extracts showed various types of antimicrobial activity on the microorganisms tested. The plant extract was prepared in the methanol solvent. The extract except *S indica* over two bacterial strains (*S. aureus* and *S. enteridi)* presented the lowest ZOI compared to the antibiotic standard (tetracycline) and can be the source of new antibiotic compounds. Further work is needed to purify the secondary metabolites from the extracts studied in order to test specific antibacterial activity. This study revealed that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms and safe alternative to treat infectious diseases.

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