World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

PHYTOCHEMICAL SCREENING AND IN-VITRO EVALUATION FOR ANTIMICROBIAL ACTIVITY OF BAUHINIA PURPUREA L. ETAHNOLIC EXTRACT

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Article Received on 06/05/2020 **Article Revised on 27/05/2020** **Article Accepted on 17/06/2020**

ABSTRACT

Aim of this study was to evaluate antimicrobial activity and perform its phytochemical analysis and screening of the leaves of Bauhinia purpurea L .in the given study. Ethanolic extract of leaves of Bauhinia purpurea L. was tested against standard bacterial species - Staphylococcus aureus, Bacillus subtilis and fungal species Aspergillus niger, Trycophyton rubrum. In vitro antimicrobial test was performed by agar well diffusion method on Nutrient agar media and Sabouraud dextrose agar. Minimum Inhibitory Concentration (MIC) and relative percentage of inhibition (%) test was performed by Modified agar well diffusion method. Ethanol extract showed significantly inhibitory effect compared to marketed antibiotic formulation of Amikacin and marketed antifungal formulation of Fluconazol on tested organism and fungal species. Ethanol extract shows a broad spectrum of antimicrobial activity as it inhibited Gram positive bacteria (S.aureus and B.subtilis) and fungal species (A.niger, T.rubrum). With respect to phytochemical analysis and screening by using spectroscopic technique we easily evaluate Ethanolic extract characters.

KEYWORDS: Bauhinia purpurea L, Antibacterial, Antifungal, Minimum Inhibitory Concentration.

INTRODUCTION

Nature has been a source of medicinal agents. Infectious disease is one of the major causes of health hazard in humans and animals. Such types of infections are caused by various pathogenic bacteria, virus and fungi. Also severity of infection depends on virulence power of microbes. In past few years scientist found that human pathogens have been reported to acquire resistance toward the common drugs. Drug resistant microbes are highly lethal and they increase the severity of infection in patient. Antimicrobial research always looks for new potent antimicrobial drugs from alternative sources. Medicinal plants proved to be a major source of new drug discovery.[1]

India,China,Nepal, and other Asian countries used many plants and plant derivatives to cure various diseases in traditional medicinal system, most recently western countries also concentrating on medicinal plants for the discovery of new chemotherapeutic compounds B.purpurea L. is a flowering plant of the (Family-Fabaceae) native to south China(Hong Kong) and southeast Asia. In India it is habitat of Sub Himalayan tracts, Uttar Pradesh, West Bengal and Central and South India. Almost all parts of the plant such as,leaves,flowers and stem bark of this plant carry many medicinal properties and used in traditional medicinal system for the treatment of several diseases.[2,3]

B.purpurea L. showed the presence of Phenolic groups, Flavonoids, Saponins, Hydroxyl flavones, Alkaloids and Tannins as major phytochemical constituent. Ethanol extract of bark shows antitumor, antimicrobial, antioxidant, anti-inflammatory, antipyretic and analgesic activity.

MATERIAL AND METHODS

Plant material

The fresh mature leaves of B.purpurea L. were collected from the natural population growing in Agashivas mountain and hills,Satara,west Maharashtra, India during Jan-Feb.2020 given plant species authentified form Kusumtai Rajarambapu Patil college,Islampur,Tal-walwa,Dist-sangli. Maharashtra, India

Microorganisms

Bacillus subtilis and Staphylococcus aureus (Gram positive) bacteria, A.niger, T.rubrum used as fungal species.

Chemicals
Ethanol, Agar, Peptone, Beef extract, Water, Sabouraud dextrose agar, Glucose, Toluene, Ethyl acetate, Formic acid,Methanol,Acetone,Chloroform,Water,n-hexane,
Silica all reagents and chemical grades are available in Rajarambapu College of Pharmacy, Kasegaon.

Preparation of crude extract
Collected leaves of respective plant species were washed properly under tap water followed by distilled water. Washed leaves were dried in hot air oven at a temperature of 40^0C. Dried leaves were powdered by using a mechanical grinder.20gms of pulverized leave material was extracted with 200ml of ethanol by using Soxhlet extraction method. After that extract was evaporated in tray dryers, hot air oven and used in method as active chemical.[4,5]
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Determination of antibacterial activity
Antibacterial activity of the extract was determined by Agar well diffusion method as described by Tagg et al., with some modifications. The bacterial suspension took into Petri plates (streak plate method) in each of these plates three wells were cut out using a standard cork borer (7mm). Using a micropipette 100 µl/borer of extract, marketed formulation and negative control was added in to different wells. After that Nutrient agar media plates were incubated for 24 hours at 37^0 C. Antibacterial activity was evaluated by measuring the zone of inhibition.[6]

Determination of antifungal activity
Antifungal activity of the extract was determined by Agar well diffusion method (Sabouraud dextrose agar). The fungal suspension took into Petri plates (streak plate method) in each of these plates three wells were cut out using a standard cork borer (7mm). Using a micropipette 100 µl/borer of extract and marketed formulation was added in to different wells. After that Sabouraud dextrose agar plates were incubated for 24 hours at 37^0 C. Antifungal activity was evaluated by measuring the zone of inhibition.[7]

Determination of Relative Percentage Inhibition
The relative percentage inhibition with respect to positive control was calculated by using the following formula-

Relative percentage inhibition of the test extract = 100× (a-b)/(c-b)

Where,
a-total area of the inhibition of the test extract
b-total are of the inhition of the solvent
c-total area of inhibition of the standard drug

Total area of the inhibition was calculated by using area = Πr2 where, r = radius of zone of inhibition)

Determination of minimum inhibitory concentration (MIC)
MIC of the plant extract was performed by Modified agar well diffusion method. The bacterial and fungal suspension was seeded on Nutrient agar media and Sabouraud dextrose agar in each of these plates three wells were cut out using a standard cork borer (7mm). Using a micropipette, 100 µl/borer of each dilution was added in wells. Bacterial and fungal plates were incubated at 37^0C for 24 hours. Antimicrobial activity was evaluated by measuring the zone of inhibition.[8]

RESULT

Antimicrobial activity
Antimicrobial activity of the Ethanolic extract of the leaves of B.purpurpurea L. on the test organism (zone of inhibition-mm) listed in **table no.1** Ethanol extract exhibits antimicrobial effect which is very good as compare with marketed Amikacin antibiotic and Fluconazol antifungal formulation. The results of the relative percentage inhibition of the test extract mentioned in **table no.2**

Fig. 1: Zone of inhibition detected on agar plate.

Table 1: Antimicrobial activity of B.purpurea L. on test organisms.

Test organisms	Zone of inhibition(mm)		
	Ethanolic extract	Marketed formulation	Solvent
Staphylococcus aureus	9.30±1.50	13.60±0.40	7.75±1.07
Bacillus subtilis	10.25±0.15	12.35±0.35	8.58±1.45
Aspergillus niger	12.45±1.00	14.32±1.75	10.70±0.62
Trycophyton rubrum	14.33±1.78	16.48±0.22	13.85±1.22

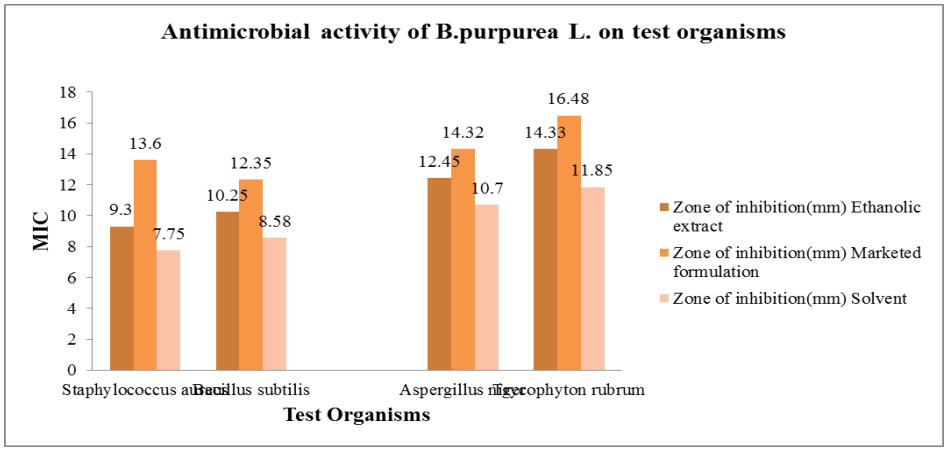

Fig. 2: Antimicrobial activity of Bauhinia purpurea L.

Table 2: Relative Percentage Inhibition on test organisms.

Test organisms	Relative percentage inhibition (%)	Standard deviation (%)
Staphylococcus aureus	12.074	17±0.225
Bacillus subtilis	89.429	95±0.360
Aspergillus niger	26.564	27±0.054
Trycophyton rubrum	16.957	21±0.728

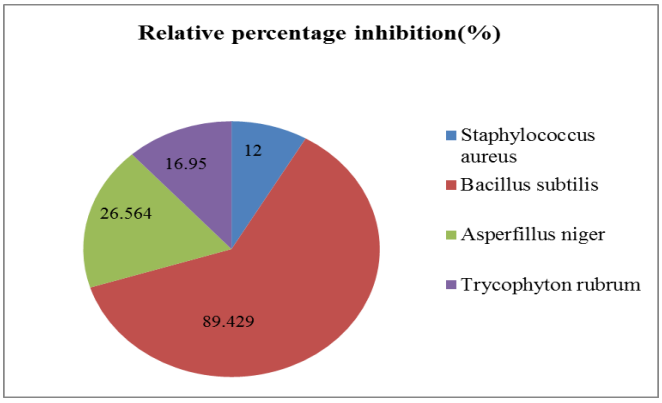

Fig. 3: Relative Percentage Inhibition.

Phytochemical analysis

FT/IR-spectroscopy

The dried leaves of B.purpurea L.were ground into fine powder using mortar and pestle. The sample pellet was placed into the sample holder and FT-IR spectra were recorded in the range 4000-650 cm^{-1} in FT-IR spectroscopy (JASCO FT/IR-4600).

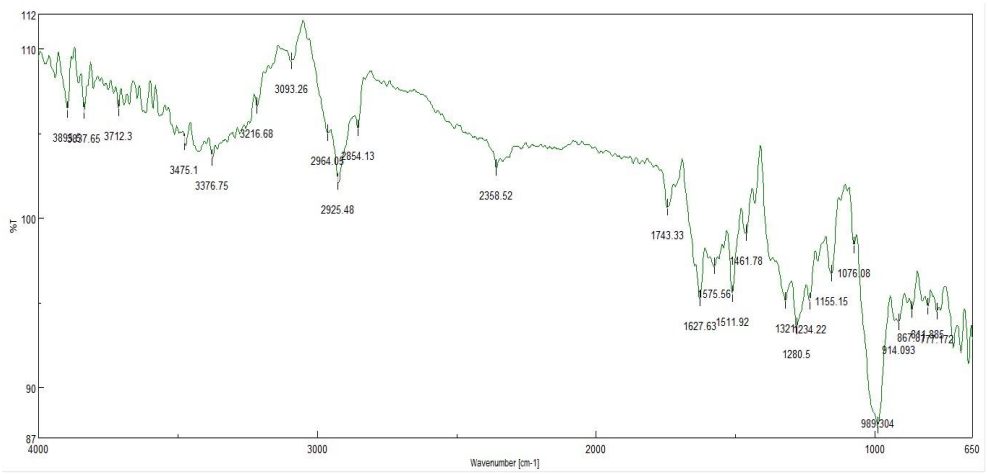

Fig. 4: FT/IR spectra of Bauhinia purpurea L.

TLC

Introduction- Thin layer chromatography is a chromatography technique used to separate non-volatile mixtures.TLC performed on sheet of glass which coated with a thin layer of adsorbent material, usually silica gel, aluminum oxide, and cellulose. This layer of adsorbent is known as the stationary phase and running phase known to be mobile phase prepared by selective solvent.

In this given work we use two solvent mixture mobile phase to separate active constituents from Ethanolic extract of bauhinia purpurea L.

1. Toluene: Ethyl acetate: Formic acid(8:1:1) *Rf value=0.315
2. Acetone:n-hexane:Toluene(3:4:3) *Rf value=0.375

Retardation/retention factor (Rf-value) - In chromatography the retardation factor is the fraction of an analyte in the mobile phase of a chromatographic system. It can be defined as the ratio of the distance traveled by the center of a spot to the distance traveled by the solvent front.

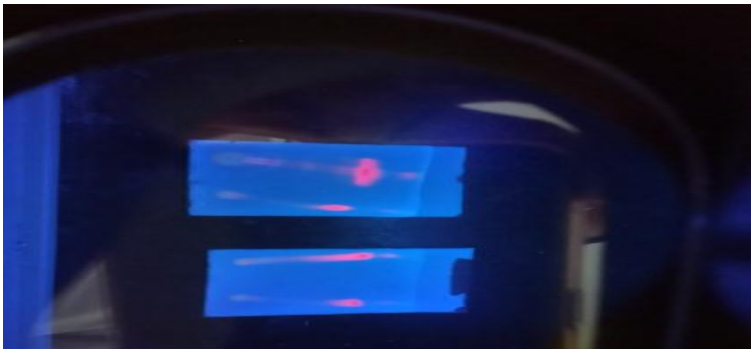

Fig. 5: TLC plate showing spot of selected solvent sample.

Phytochemical Screening.

Sr. No.	Presence/absence of bioactive components	Name of the extract			
		Chloroform	Acetone	Methanol	Water
1	Alkaloids	-	-	+	-
2	Anthraquinones	+	+	-	+
3	Coumarin	-	-	+	-
4	Flavonoids	-	+	+	-
5	Saponins	+	+	+	-
6	Tannins	-	+	+	+
7	Phenols	+	-	+	-
8	Steroids	+	-	+	-

DISCUSSION

Phytochemical analysis (preparative TLC, FT/IR-Spectoscopy) of the leaves of B.purpurea L.was screened for antimicrobial analysis. Results of the antimicrobial study of leaves of the B.purpurea L. contain Phenolic groups, Flavonoids, Saponins, Alkaloids and Tannins as major phytochemical groups. These phytochemical may play an important role in antimicrobial properties.

In this in-vitro study Ethanolic extract as compare with Amikacin and Fluconazol shows very good antibacterial and antifungal activity on bacteria and fungus by Agar well diffusion method. This study concludes that B.purpurea L. is a valuable medicinal plant possessing a broad range of antimicrobial activity and can be explored for isolation of natural antimicrobial compounds.

CONCLUSION

Based on in-vitro study perform in this work, we conclude that leaves of B.purpurea L.possess significant amount of antimicrobial property against a wide range of microorganisms and fungal species. Results also reports the presence of various phytochemical may play key role in the medicinal value of this plant.

ACKNOWLEDGEMENT

Authors are highly Acknowledge the help of laboratory staff of Rajarambapu College of Pharmacy, Kasegaon. For providing necessary equipment required for research work. Also we are highly Acknowledge the help and guidance of Principal and vice principal of RCP, Kasegaon.

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