

EVALUATION OF ANTIMICROBIAL AND ANTIFUNGAL STUDIES OF THE SEED OIL OF BASELLA RUBRA LINN

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ABSTRACT

A number of natural products have already been proved to exhibit antimicrobial activity against a number of bacteria (both gram positive and gram negative) fungi etc.. This paper contains all the informations regarding the antibacterial and antifungal studies carried out from the seed oil of Basella rubra. The antimicrobial activities of aqueous, ethanolic and petroleum ether extracts of the leaves of Basella rubra were evaluated in the present study by measuring the inhibition zones using Cup plate Diffusion method.

KEYWORDS: Antibacterial, Antimicrobial, Basella Rubra, Cup Plate Diffusion Method

INTRODUCTION

A number of natural products have already been proved to exhibit excellent anti-microbial utility against a number of bacteria (both gram positive and gram negative), fungi etc

In south-western part of Nigeria, Psidium guajava and Mangifera indica are commonly used for herbal preparation in the treatment of toothache, gastro intestinal disorders, dysentery, diarrhea, sore gums and sore throats. This has therefore, led to the investigation of the antimicrobial activities of methanolic extracts of P. guajava and M. indica. The extracts exhibited antimicrobial activities at a concentration of 20 mg/ml. The zones of inhibition exhibited by P. guajava extract ranged between 12 mm and 30 mm while that M. indica varied between 11 mm and 28 mm.

In the study performed on six endemic plant species, antimicrobial activity was observed in Campanula trachelium subspecies Lyrata and Abies nordmanniana subsp. bornmuelleriana plants (Singh et al., 2010). The minimum inhibitory concentration of C. lyrata subspecies Lyrata (leaf and flower extract) was found to be 20 mg/ml for Baccillus subtilis and 14.5 mg/ml for Staphylococcus aureus, and the minimum inhibitory concentration (MIC) of Abies nordmanniana subsp. bornmuelleriana (leaf) extract was found to be >314 mg/ml for B. subtilis and when minimum bactericidal concentration (MBC) results were evaluated, it was observed that the plant extracts had bactericidal effects (Rajkumar et al., 1973).

LITERATURE REVIEW

Phytochemical screening of the leaves and roots of Cassia alata (Linn) revealed the presence of some bioactive components, which have been linked to antimicrobial properties (S. et al., 1996). The effects of water, methanol and chloroform extracts on some pathogenic Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa and Proteus mirabilis showed that the parts can be used to treat infection caused by these bacteria. S. aureus, S. pyogenes & P. mirabilis were more susceptible, while E. coli & P. aeruginosa were less sensitive. The effectiveness of the crude extracts was enhanced at elevated temperatures and at near neutrality pH values, which attest to its use in traditional medicine to treat skin, urinary tract and gastrointestinal infection (Sathe et al., 1999).

The antibacterial effect of some selected Indian medicinal plants was evaluated by Parekh and Chanda on bacterial strains like *Bacillus cereus* ATCC11778, *Staphylococcus aureus* ATCC 25923, *Enterobacter aerogenes* ATCC 130048, *Escherichia coli* ATCC25922 and *Klebsiella pneumoniae* NCIM 2719. The Most susceptible gram – Positive bacteria were *B.cereus*, while the most susceptible gram - negative bacteria were *K. Pneumonia*.

Keeping all these into account the antibacterial and antifungal studies were carried for the seed oil of *Basella rubra* (Sharma et al., 1961). The antimicrobial activities of aqueous, ethanolic and petroleum ether extracts of leaves of *basella rubra* were evaluated in the present study by measuring the inhibition zones using Cup Plate Diffusion method. The inhibition zones were significantly different ($P < 0.001$) in each plant extract. The ethanolic extract showed maximum activity with zone of inhibition (14.3 ± 1.82 mm) and petroleum ether (5.6 ± 0.62 mm) at a concentration of 50 mg/ml. Ciprofloxacin was used as the standard drug having zones of inhibition was in the order *E. coli* (12.57 ± 0.60), *A. Niger* (11.68 ± 0.71), *V. cholera* (11.42 ± 0.60), *S. aureus* (10.71 ± 0.46), *S.typhi* (9.80 ± 0.90), respectively with all the extracts. The extracts were not able to inhibit the growth of *P. acuginosa*.

METHODOLOGY FOR ANTI BACTERIAL STUDIES

The antibacterial studies carried out for the extracts were done systematically against four strain of bacteria (2gram positive: *Bacillus* & *Staphylococcus aureus*: 2 gram negative: *Escherichia coli* & *Vibrio cholerae*) by agar diffusion method in particular paper disc method. The standard drug (Tetracycline) solution was made in chloroform in 200 µg/ml. (Pelezar et al., 1993; Panda et al., 2005).

The nutrient agar media was first prepared, whose composition is given below. The prepared media was sterilized by autoclaving at 121°C (15lb/sq. inch) for fifteen minutes. The slants were prepared and the test organisms were subcultured in the fresh media, which were then incubated for 24 hr. at $37 \pm 1^{\circ}\text{C}$. Then they were prepared by transferring a loopful of stock culture to sterilized nutrient broth taken in the test tubes, which were incubated at $37 \pm 10^{\circ}\text{C}$ for 18 hrs before the experiments were carried out (Singha et al., 2007).

Petri dishes, test tubes, flasks plugged with cotton were first sterilized in Hot Air Oven for one hour. Fresh sterilized nutrient agar media for Bacteria and Yeast was again prepared, which in molten condition was transferred aseptically into the sterilized petri dishes and left at room temperature to be solidified, 2 ml of included nutrient broth was then transferred in each of the petri dishes aseptically. The broth was spread uniformly throughout the entire media.

In each of the plates, paper disc of 6mm diameter soaked with the standard solution (Tetracycline), extract, the oil and the solvent (chloroform), were placed aseptically. The plates were kept undisturbed for atleast 2 hrs for room temperature to allow diffusion of the solution properly into the nutrient media. After incubation of the plate $37 \pm 1^{\circ}\text{C}$ for 24 hrs. The diameter of zone of inhibition surrounding each of the discs was measured. All the experiments were carried out in triplicate.

Composition of Nutrient Agar Medium of Bacteria and Yeast

Peptone	60g
Beef extract	15g
Sodium Chloride	5g
Dextrose	10g
Agar	25g
Distilled Water	100ml

Final pH after sterilization6.5 to 6.6

METHODOLOGY FOR ANTI FUNGAL STUDIES

Antifungal studies were carried out for one strain of *Candida albican* bY Agar diffusion method, in particular paper disc method. The zones of inhibition were observed and recovered. The standard drug solution was made in DMF (200 µg/ml). The test organism was subcultured in fresh Sabaraud Dextrose Agar (SDA) media, composition of which is given below. The SAD media prepared was first sterilized at 121⁰C (15lb/sq. inch) for 15 minutes by autoclaving (Kalita et al., 2006).

The slant was prepared by sterilized SDA media, which was then subcutured with the test organism. The slant was then maintained at 22-25⁰C for 48 hrs. from this loopful of fungus strain was then transferred aseptically in nutrient broth media and again maintained at 22-25⁰C for 48 hrs.

The sterilized SDA media was then placed in the sterilized petri dishes in molten condition and were allowed to solidity at room temperature. The inoculated broth of 2 ml was then spread uniformly and aseptically over the prepared plates. In each plate paper disc of 6 mm diameter soaked in the standard drug solution (Amphoterican B), oil and the solvent were placed aseptically accordingly (Singha et al., 2007).

The prepared plates were then maintained at 22-25⁰C for 48 hrs and then the diameter of the zone of inhibition surrounding each of the disc was measured. All the experiments were carried out in Triplicate.

COMPISITION OF MEDIUM OF FUNGUS

SABARAUD DETROSE AGAR (SDA) MEDIA

Dextrose	40mg
Peptone	10mg
Agar	25 mg
Distilled water	100mg

RESULTS AND ANALYSIS

The antimicrobial activities of aqueous, ethanolic and petroleum ether extracts of the leaves of *Basella rubra* were evaluated in the present study by measuring the inhibition zones using Cup Plates Diffusion and Diffusion method.

In the Cup Plate Diffusion method, the inhibition zones were found significantly different ($P < 0.001$) in each plant extract. The ethanolic extract showed maximum activity with zone of (14.3±1.82mm) against *E.coli* followed by aqueous extract (13.4±1.2mm) and petroleum ether (5.6±0.62 mm) at a concentration of 50 µg/ml

Ciprofloxacin was used as the standard drug having zones of inhibition (17 ± 0.34 mm) against *E.coli* and 19 ± 0.18 mm against *A.niger*. Microbial inhibition was in the order *E.coil* (12.57 ± 0.99), *A. niger* (11.68 ± 0.71), *V.cholera* (11.42 ± 0.60), *S.aureus* (10.7 ± 0.46), *S. typhi* (9.80 ± 0.90), respectively with all the extracts (kalita et al., 2006). The extracts were not able to inhibit the growth of *P. aeruginosa*.

Table 1 : Results of Antimicrobial Studies (using Cup Plate Diffusion method)

	Bacillus Pertisis	Staphylococcus aureus	Escherichia coil	Vibrio cholarae	Yeast	Candida albilians
	9 (>1500)	10 (>1500)	11 (1500)	10 (1500)	11 (1450)	15 (900)
Tetracycline >	23 (30)	24 (250)	24 (27)	23 (30)	25 (200)	-
Amphotericin] B >	-	-	-	-	-	16 (55)

The results of the antimicrobial studies by Disc diffusion method are presented in Table below:

Table 2 : Consolidated results of the Antimicrobial Analysis

Sl. No.	Microbe	Standard	Solvent (CHCL ₃)	SRS6	SRS	SRS12	SRS14
1	Streptococcus facealis	40mm	Nil	18	11	16	10
2	Bacillus	32mm	Nil	10	13	Nil	Nil
3	Klebsila pneumoae	25mm	Nil	14	14	12	11
4	E. coli	35mm	Nil	15	16	10	10
5	Proteus vulgaris	40mm	Nil	15	13	12	12
6	Pseudomonas aeruginasa	25mm	Nil	18	16	24	18
7	Candida albicans	22mm	Nil	14	15	10	10
8	S.aureus	22mm	Nil	Nil	12	Nil	Nil

The effect of the residual solvent (chloroform) was checked by using dicks soaked in the solvent and then dried.

The photograph of the culture plate is presented in figure below:

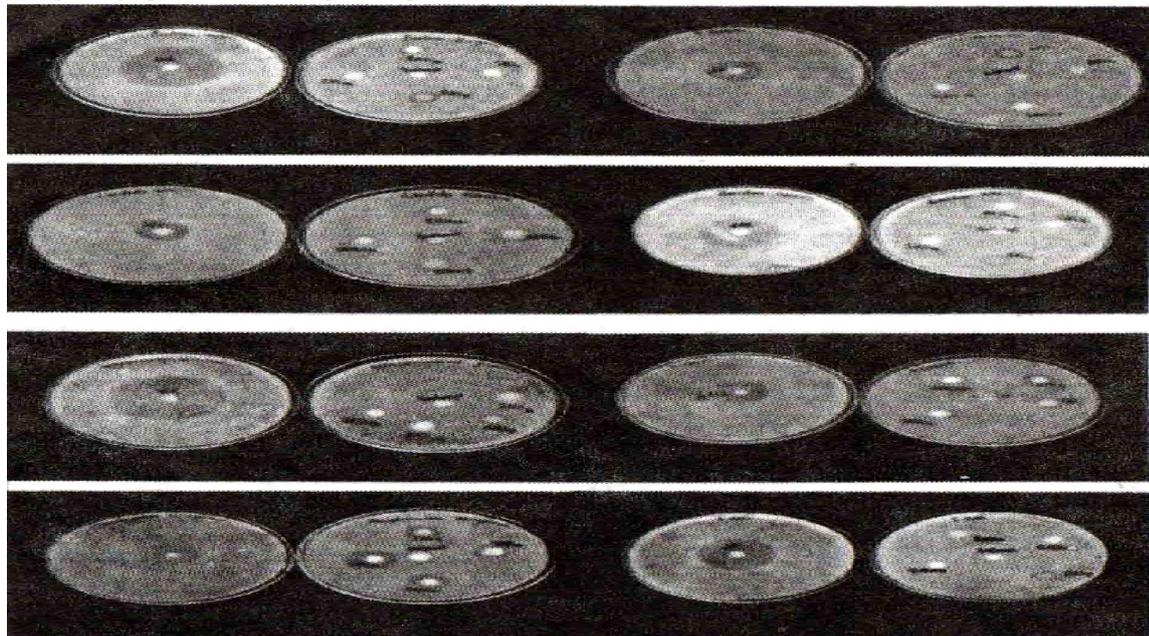


Figure 1: Photographs of the antimicrobial assay of plant materials

CONCLUSION

The material exhibited very good inhibitory effect against *Streptococcus faecalis* & *Bacillus subtilis* and lesser activity against *Klebsilla pneuginosa* relative to standard ciprofloxacin and clotrimazole. The solvent chloroform has no antimicrobial activity as such.

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