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# PRELIMINARY PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTIMICROBIAL ACTIVITY OF BUCHNANIA LANZAN (CHIRONJI) FROM CHHATTISGARH

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## ABSTRACT

The present study primarily aims to carry out a preliminary phytochemical screening of *Buchnania lanzan* so as to detect the major class of compounds present. Antimicrobial activity was carried out using sequential extracts of solvents with varying polarity; Petroleum ether, Chloroform, Methanol Ethanol and Water respectively. The phytochemical analysis had showed the presence of saponins, flavanoids, steroids, cardiac glycosides, carbohydrate, tannins and phenolics. All solvent extracts were screened for antimicrobial potential against three bacterial species (*Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus*) two fungal species (*Aspergillus Niger* and *Penicillium sp*.) by disc diffusion method. Extract Showed maximum antibacterial activity against all organisms tested.

**KEYWORDS:** *Buchanania lanzan*, Phytochemicals, Flavenoid, Tannin, Phenol, Antimicrobial activity.

## INTRODUCTION

*Buchnania lanzan* Spreng syn. *B. latifolia* Roxb is also known as char, piyal, achar or chironji belongs to the family Anacardiaceae. It was first described by Francis Hamilton in 1798. (Siddiqui *et al.*,2014). It is a medium-sized deciduous tree, growing to about 50 ft tall, subtropical, underutilized/underexploited popular edible nut fruit, eaten raw or roasted and also used in making dessert. It is considered to be native to India and is commonly found in the dry forests of Jharkhand, Madhya Pradesh, Chhattisgarh, Varanasi and Mirzapur districts of Uttar Pradesh. This multipurpose tree provides food, fuel, fodder, timber and medicine to

the local community. (Dwivedi *et al.*,2012). Chironji tree is a commercially important tropical plant, makes an important contribution to the tribal economy of Chhattisgarh states along with two other species namely *Madhuca indica* (Mahua) and *Diospyros melanoxylon* (Tendu). Chironji tree has great medicinal value and used as a drug of the ayurveda and the Unani system of medicine. Chironji is known to have tonic, cardio tonic, and the parts of the plant are used for the treatment of various disorders (Kumar J. 2012) these species play very important role in the socio-economic condition of tribal population of this state. Secondary metabolites are responsible for medicinal activity of plants. Plant products have been part of phytomedicines since time immemorial. Knowledge of the chemical constituents of plants is desirable because such information will be of value for the synthesis of complex chemical substances.

## MATERIALS AND METHODS

### Plant Material

Fresh leaves of Chironji trees were collected from Horticultural nursery of Agricultural University campus during the month of December, 2013. Phytochemcial analysis was carried by Soxhlet Extraction using successive solvents such as Water, Petroleum ether, Chloroform, Methanol and ethanol in increasing polarity.

The leaves were cleaned with running tap water, then air dried under shade, dried leaves were ground and sieves into a fine powder. The powder was kept in small plastic bags maintained at $4^0$c for Soxhlet's extraction. 20 gm of the powder was filled in the thimble and extracted successively with selected solvents and allowed refluxing for 5 hrs. These extracts were concentrated using rotary flash evaporator (Rotava) and were weighed to determine the yield and stored and preserved at 4 $^oC$ in airtight bottle until further use.

### Phytochemistry of the Buchnania Lanzan Leaves

Phytochemcial tests were carried out on all extract using standard methods for identification of Phenols, Alkaloids, Tannins, Flavonoids, Steroids, Saponins, Reducing sugars and Cardiac glycoside and Anthraquinones as described by following the standard method of Khandelwal (2000). A. Poongothai (2011).

1. **Flavonoids**

The extract (5 ml) was added to a concentrated sulphuric acid (1 ml) and 0.5g of Mg. A pink or red coloration that disappear on standing (3 min) indicates the presence of flavonoids.

2. **Tannins**

About 1 ml of the extract was added in 2 ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (cathechic tannins) or a blue-black (Gallic tannins) coloration.

3. **Saponins**

To 1 ml of aqueous extract was added few volume of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth for 20 min.

4. **Alkaloids**

10 ml of concentrated etheric solution, the dry residue was added to 1.5 ml HCl (2%) acid solution. After that, 1 to 2 drops of Mayer's reagent and Wagner was added, and the yellow-white precipitate indicates the presence of the alkaloid base.

5. **Sterols And Steroids**

Sterols and steroids were sought by the reaction of Liebermann. Ten (10 ml) ml of extract was evaporated. The residue was dissolved in 0.5 ml of hot acetic anhydride; added 0.5 ml of the filtrate chloroform. Treated with the reagent of Libermann Burchardt. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

6. **Anthraquinones**

**Borntrager's Test:** About 5 ml of extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

7. **Carbohydrate**

**Molisch's Test:** Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

8. **Proteins**

**Ninhydrin Test:** To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue colour indicates the presence of amino acid.

**Quantification of Total Phenol, Flavenoid and Tannin Content of *Buchnania Lanzan* Leaves**

Quantitative analysis of total Flavenoid, total Tannin and total Phenol content was determined by spectrophotometric method as per the modified methodology given by (Pattnaik *et al.*, 2013). Rutin and Gallic acid (1mg/ml) were used as standards as positive control for determination of total Flavonoid, Tannin and Phenol content. (Chavan, *et al.*, 2013)

**Total Tannin content was expressed as**

| Tannins = Total phenol - phenol conc. in the solution analyzed after PVP precipitation |
|---|

**Test Microorganisms**

Three different bacterial cultures like *Escherichia coli (*ATCC No. 25922), *Pseudomonas aeruginosa*, (ATCC No 27853) and *Staphylococcus aureus (*ATCC No.25923) (Kwikstik *Microbiologics)* and two fungal strains like *Aspergillus* and *Penicillium* were obtained from the Department of Biotechnology, Degree Girls College, Raipur Chhattisgarh, India.

**Growth Medium and Inoculum Preparation**

The media used for antibacterial test was nutrient Agar/Broth of High Media Pvt Ltd Mumbai, India. The bacterial and fungal strains were inoculated in liquid medium (nutrient broth) and incubated at 37°c for 8hrs and further used for the test (105- 106 CFU/ml). These suspensions were prepared immediately before the test was carried out.

**Antimicrobial Testing**

The disc diffusion assay was performed to determine the growth inhibition of bacteria by leaf extract. A diluted culture (0.2ml) was spread over nutrient agar plates using sterile glass L-rod. Around 0.3ml of the each extract was applied per filter paper disc and was allowed to dry before placed on the top layer of the agar plates. Each extract was tested in triplicate and the plates were incubated at 37°c for 24 hours for bacteria and for fungus the zones of inhibition were noted.

**Sensitivity of the Microbes to Standard Antibiotics**

The Standard antibiotics such as Penicillin-G and Streptomycin (10 Units/disc Himedia, Mumbai) were used for determining the antibacterial activity and Fluconazole (10mg, Galfa) was selected as standard for antifungal activity of the experimental plant samples. The

microbe's sensitivity to the antibiotics was analyzed by the zone of inhibition against the selected pathogens.

## RESULT AND DISCUSSION

**Table 1: Determination of Percentage Yield of Plant Extracts in Different Solvent System.**

| Plant name | Solvent | | | | |
|---|---|---|---|---|---|
| | Chloroform | Methanol | Pet.Ether | Ethanol | Water |
| Buchnania Lanzan | 2.8 | 3.8% | 3.0% | 3.5% | 2.5% |

**Table 2. Phytochemical Screening of Leaves Extract of Buchnania Lanzan.**

| S. No | Test | Solvents | | | | |
|---|---|---|---|---|---|---|
| | | Chloroform | Methanol | Pet. Ether | Ethanol | Water |
| 1 | Alkaloids | - | - | - | - | - |
| 2 | Tannin | + | + | + | + | + |
| 3 | Saponin | - | + | - | + | + |
| 4 | Glycoside | + | + | + | + | + |
| 5 | Steroids | + | + | + | + | + |
| 6 | Flavenoids | + | + | + | + | + |
| 7 | Phenol | + | + | + | + | + |
| 8 | Carbohydrate | + | + | + | + | + |
| 9 | Protein | - | - | - | - | - |
| 10 | Anthraquinone | - | - | - | - | - |

**Table 3: Quantitative estimation of total Flavenoid, Tannin and Phenol content by Spectrophotometric method**

| Solvent system | Total Flavenoid content (mg/ml) | Total Tannin content (mg/ml) | Total phenol content (mg/ml) |
|---|---|---|---|
| Methanol | 1.13 | 1.19 | 1.41 |
| Ethanol | 1.10 | 0.34 | 1.04 |
| Chloroform | 1.09 | 0.28 | 0.92 |
| Pet.Ether | 1.12 | 0.88 | 1.02 |
| Water | 1.11 | 1.01 | 0.81 |

**Table 4: Antibacterial activity of *Buchanania lanzan* (Chironji) extracts by measuring zone of inhibition diameter (in mm).**

| Test pathogen | Solvent | Zone Of Inhibition | | |
|---|---|---|---|---|
| | | Extract (mm) 5 µl/ disc | Penicillin (mm) 10 units/disc | Streptomycin (mm) 10 units/disc |
| Escherichia Coli | Methanol | 8±0.33 | 16±0.33 | 15±0.66 |
| | Ethanol | 9±0.33 | | |
| | Chloroform | 4±2.9 | | |
| | Pet.ether | 7±0.33 | | |
| | Water | 3±0.6 | | |
| Pseudomonas Aeruginosa | Methanol | 7±0.00 | 17±0.51 | 16±1.15 |
| | Ethanol | 6 ±9.3 | | |
| | Chloroform | 8±0.67 | | |
| | Pet.ether | 6±0.11 | | |
| | Water | 4±1.12 | | |
| Staphylococcus Aureus | Methanol | 8±0.57 | 19±0.33 | 18±0.88 |
| | Ethanol | 9±57 | | |
| | Chloroform | 6±0.57 | | |
| | Pet.ether | 5±54 | | |
| | Water | 2±0.12 | | |

**Table 5: Antifungal activity of *Buchanania lanzan*(Chironji) extract by measuring zone of inhibition diameter (in cm).**

| Test pathogen | Solvent | Extract(mm) | Fluconazole (mm) |
|---|---|---|---|
| Aspergillus sp. | Methanol | 10.3±0.35 | 20.1±0.62 |
| | Ethanol | 7.4+0.99 | |
| | Chloroform | 8.8+2.9 | |
| | Pet.ether | 6+1.2 | |
| | Water | 3.9+3.7 | |
| Peniciilum sp. | Methanol | 16.9+0.41 | 21.5±0.32 |
| | Ethanol | 12.4+0.76 | |
| | Chloroform | 9+0.44 | |
| | Pet.ether | 9.89+1.3 | |
| | Water | 3.12 | |

The preliminary phytochemical analysis of leaves of *Buchnania lanzan* using successive solvents such as Water, Petroleum ether, Chloroform, Methanol and ethanol in increasing polarity. Table 1 shows the percentage yield of plant extracts in different solvent system on the basis of Extraction. According to the Table 2 result has been revealed that the leaves extract of *Buchnania Lanzan* contain Steroids, Carbohydrate, Flavenoid, Phenols, Glycoside and Tannins. Protein, and Alkaloids were totally absent in all the solvent extract, while presence of Saponin was found in Methanolic, Ethanolic and aqueous extract of *Buchnania Lanzan* Leaves. Phytochemical analysis results were similar to the results of Shalini Kapoor

Mehta (2010).The results for Quantitative analysis of phytoconstituents like Phenol, Tannin and Flavenoids present in Table 2 was done with the published methods.( Ainsworth and Gillespie, 2007).

In the present investigation, it was found that Buchnania Lanzan has potent antibacterial activity. The above results show that the leaf extract of *Buchnania Lanzan* having antibacterial and antifungal activities and this was comparable to that of the standard Antibiotics like ampicillin Penicillin-G and Streptomycin (10 Units/disc Himedia, Mumbai) and Fluconazole (10mg, Galfa) as shown in Table 3 and 4.Ethanolic and Methanolic extract of leaves shows high antimicrobial activity. Chloroform and Aqueous extract of *Buchnania Lanzan was* less sensitive to the bacteria as well as fungus shows in Table 3 and 4. The methanol leaf extract of *Buchanania lanzan had* more antibacterial properties than other solvent extract. The maximum antibacterial activity was observed in *E. coli* followed by *P. aeruginosa* and *S. aureus*, while in case of antifungal activity Aspergillus spp. was found to be more sensitive than Penicillium. The similar antibacterial results were obtained by Verma *et al.*, (2010) has reported that the methanolic extract of leaves of *Buchanania lanzan* Spreng was more effective against gram negative bacteria (*Vibrio chlorae* and *Klebsiella pneumonae*). Manjunath and Mithun (2011) have also found that the methanolic extract of leaves of *Buchnania lanzan* was more significantly active against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* in comparison to extracts of *Buchanania lanzan* leaves in petroleum ether, chloroform and water.

## CONCLUSION

In conclusion it may state that *Buchnanania Lanzan* leaf extract is rich source of Phytochemcials like Tannin, Phenol, Steroids, Glycosides, Flavenoids etc. plant extracts in different solvent system confirms the presence of diverse group of phytochemicals. These chemical constituents which induces the biological activities. Antibacterial substances like Saponins, Glycosides, Flavenoids, Tannin, and Phenol etc, are found to be distributed in plant leaf extract. Antimicrobial study of this plant revealed that the *Buchnania lanzan* leaf extract potentially valuable for the future as a antimicrobial drugs.

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