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# *IN VITRO* ANTIMICROBIAL ACTIVITY NUTRITIONAL PROFILE OF MEDICINAL PLANT OF GARHWAL, HIMALAYA

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**ABSTRACT**

The present study is aimed at evaluating the *in vitro* antibacterial and antifungal activities of petroleum ether, chloroform, ethyl acetate, acetone, methanol, ethanol and water extracts of medicinal plant, *Pyrus pashia* were tested against (different gram positive and gram negative) ten bacterial strains and three fungal strains using by disc diffusion method. The different fractions of bark, fruit and leaf of *Pyrus pashia*, the ethanolic bark extracts of *Pyrus pashia* showed significant activity 17±1mm, 15±1mm and 14±1mm against *Klebsiella pneumonia*, *Shigella flexneri* and *Escherichia coli*. The medicinal plant fruit contain ash value, (total ash 1.10 ± 0.05%), moisture 60.36±0.25%, crude fat 1.62±0.20% and crude fiber 5.26±0.05%, extractive values were studied fresh part weight. The preliminary phytochemical analysis test showed the presence of carbohydrates (28.38±0.12%) and glycosides, alkaloid, flavonoids, saponins, tannins, unsaturated triterpenoids and sterol, resin.

**INTRODUCTION:** Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs [1]. The less availability and high cost of new generation antibiotics necessitates looking for the substances from alternative medicines with claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in different traditional literatures [2, 3, 4].

The multidrug resistant strain of many microorganisms has revealed exploration of alternative antimicrobial agent. Medicinal plants have become the focus of intense study in terms of validation of their traditional uses through the determination of their actual pharmacological effects. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases. Fruits and vegetables are normally contaminated with microorganisms, this situation have forced scientists to search new antimicrobial substances in various sources. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants [5].

**MATERIAL AND METHODS**:

**Plant Material:** The fresh parts of fruit, bark, root and leaf of *Pyrus pashia*, was collected from adjoining area of Ghat city (Dist- Chamoli, Uttarakhand) in the month of August. The plant was authenticated by botanist Dr. R. D. Guar, Department of Botany; H. N. B. G. U. Srinagar Garhwal.

**Preparation of plant Extract**: The plant material was separated into its selected parts (bark, leaf, root and fruit) air dried ground to moderately fine powder and

Soxhlet extracted with increasing polarity solvent (Petroleum ether, chloroform, ethyl acetate, acetone, methanolic, ethanolic and water) [6]. Each extract was evaporated to dryness under reduce pressure using rotary evaporator. The coarse powder of fruit bark and root was subjected to successive hot continuous extraction with various solvent each time before extracting with next solvent the powdered material will be air dried (weight of crude extract 100gm). The various concentrated extracts were stored in air tight container for further studies.

**Media**: Nutrient broth, Nutrient agar, Muller Hinton agar, Malt extract broth and Sabouraud dextrose agar, Alcohol, Hydrochloric acid, alcohol, and sulphuric acid, Distilled water etc all product of Himedia Laboratories Mumbai (India) were used in this study.

**Bacterial Strains**: Ten bacterial strains were used namely *Escherichia coli, Klebsiella pneumoniae, Enterobacter gergoviae, salmonella entericatyphim, shigella flexneri, Staphyloccus aureus, staphyloccus epidermidis, streptococcus pyogenes*, and *Bacillus cereus*. The bacterial strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India (Customer no. 3921).

**Fungal Strains**: Three fungal strains were used namely *Candida albicans, Aspergillus flavus* and *Aspergillus parasiticus*. The fungal strains were supplied by the Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Chandigarh, India.

**Antibacterial Assay**: The disc diffusion assay methods were used to determine the growth inhibition of bacteria by plant extracts [7, 8].

Diluted bacterial culture (100µl) was spread over nutrient agar plates with a sterile glass L-rod. 10mg/ml and 50mg/ml of the each extracts were applied to each filter paper disc (Whatmann No. 1, 5 mm diameter) and allowed to dry before being placed on the agar plate. Each extract was tested in triplicate (3 discs/ plate) and the plates were inoculated at 37°C for 24 h. After incubation, the diameter of inhibition zones was measured with a caliper.

**Antifungal Assay**: The antifungal activity was tested by disc diffusion method [9, 10]. The Sabouraud dextrose agar plates were each similarly seeded with each fungal strain The 24 hrs. broth culture of each bacterium and 7 days inoculated fungus culture were used to seed sterile Sabouraud dextrose agar at 45°C respectively, and fungal plates were incubated at 25-28°C for 7 days after which diameter of zones of inhibition were measured. Each disc filled with extract.

**Nutritional & Mineral Assay**: The number of water molecule is contain % of moisture, Pt. ether and hexane soluble part is called crude fat and the non soluble part of acid- base medium is called crude fiber (cellulose and lignin), and mineral estimated by flame photometry [11, 12].

**RESULT AND DISCUSSION:** Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the in vitro antimicrobial activity assay [13]. The results of antibacterial, antifungal, nutritional value and phytochemical screening activity, **table 1, 2, 3,** and **4**, reveals that antibacterial, antifungal, nutritional, and phytochemical screening activity of bark and fruit explants of *Pyrus pashia* was evaluated against ten bacterial and three fungal pathogenic strains (**fig. 1, 2 & 3**).

**TABLE 1: ANTIBACTERIAL ACTIVITY OF TEN BACTERIAL STRAINS AGAINST *PYRUS PASHIA* PLANT EXTRACT**

| Bacterial Name | | Petroleum ether Extract | | Chloroform Extract | | Ethyl acetate Extract | | Acetone Extract | | Methanol Extract | | Ethanol Extract | | Water Extract | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genus/Species/ Subspecies | MTCC (Code) | 10 mg/ml | 50 mg/ml | 10 mg/ml | 50 mg/ml | 10 mg/ml | 50 mg/ml | 10 mg/ml | 50 mg/ml | 10 mg/ml | 50 mg/ml | 10 mg/ml | 50 mg/ml | 10 mg/ml | 50 mg/ml |
| *Bacillus cereus* | 1272 | - | - | 6 | 7 | 8 | 9 | 11 | 13 | - | 11 | 9 | 11 | 11 | 12 |
| *Escherichia coli* | 729 | - | - | 8 | 9 | 8 | 9 | - | 9 | 7 | 13 | 10 | 14 | - | 11 |
| *Enterobacter gergoviae* | 621 | - | - | 8 | 9 | 7 | 9 | 9 | 9 | 7 | 12 | 12 | 13 | - | 10 |
| *Klebsiella pneumonia* | 432 | - | - | 8 | 9 | - | 9 | - | 13 | - | 11 | 9 | 17 | 7 | 14 |
| *Salmonella entericatyphim* | 98 | - | - | 8 | 9 | - | 8 | - | 9 | 6 | 11 | 13 | 14 | - | 9 |
| *Shigella flexneri* | 1457 | - | 7 | 8 | 9 | 8 | 10 | - | 10 | - | 11 | 10 | 15 | - | 9 |
| *Staphyloccus aureus* | 902 | - | 10 | 8 | 9 | 7 | 11 | 11 | 14 | - | 11 | 11 | 12 | 10 | 11 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Staphyloccus epidermidis* | 435 | - | - | 7 | 9 | 7 | 9 | - | 10 | - | 11 | 8 | 10 | - | 10 |
| *Streptococcus pyogenes* | 1925 | - | - | - | 7 | - | 8 | - | 11 | - | 10 | 8 | 10 | 9 | 12 |
| *Escherichia coli* | 443 | - | 7 | 9 | 10 | 8 | 9 | - | 10 | - | 12 | 9 | 14 | - | 11 |

Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone

**TABLE 2: FUNGAL ACTIVITY OF THREE FUNGAL STRAINS AGAINST PYRUS PASHIA PLANT EXTRACT**

| Fungal Name | | Petroleum ether Extract | | Chloroform Extract | | Ethyl acetate Extract | | Acetone Extract | | Methanol Extract | | Ethanol Extract | | Water Extract | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Genus /Species /Subspecies** | **MTCC (Code)** | **10 mg/ ml** | **50 mg /ml** | **10 mg /ml** | **50 mg /ml** | **10 mg /ml** | **50 mg /ml** | **10 mg/ ml** | **50 mg/ ml** | **10 mg/ ml** | **50 mg/ ml** | **10 mg/ ml** | **50 mg/ ml** | **10 mg /ml** | **50 mg/ ml** |
| *Candida albicans* | 3017 | - | - | - | 7 | - | - | - | - | - | 8 | - | 8 | - | - |
| *Aspergillus flavus* | 2798 | - | - | - | 7 | - | - | - | - | - | - | - | 8 | - | - |
| *Aspergillus parasiticus* | 2796 | - | 8 | - | 8 | - | - | - | - | - | - | - | 7 | - | - |

Disc size, 5 Mm, Inhibitory zone size ±1 Mm, Mm means (millimetres) and – indicate (NIZ) No inhibitory zone

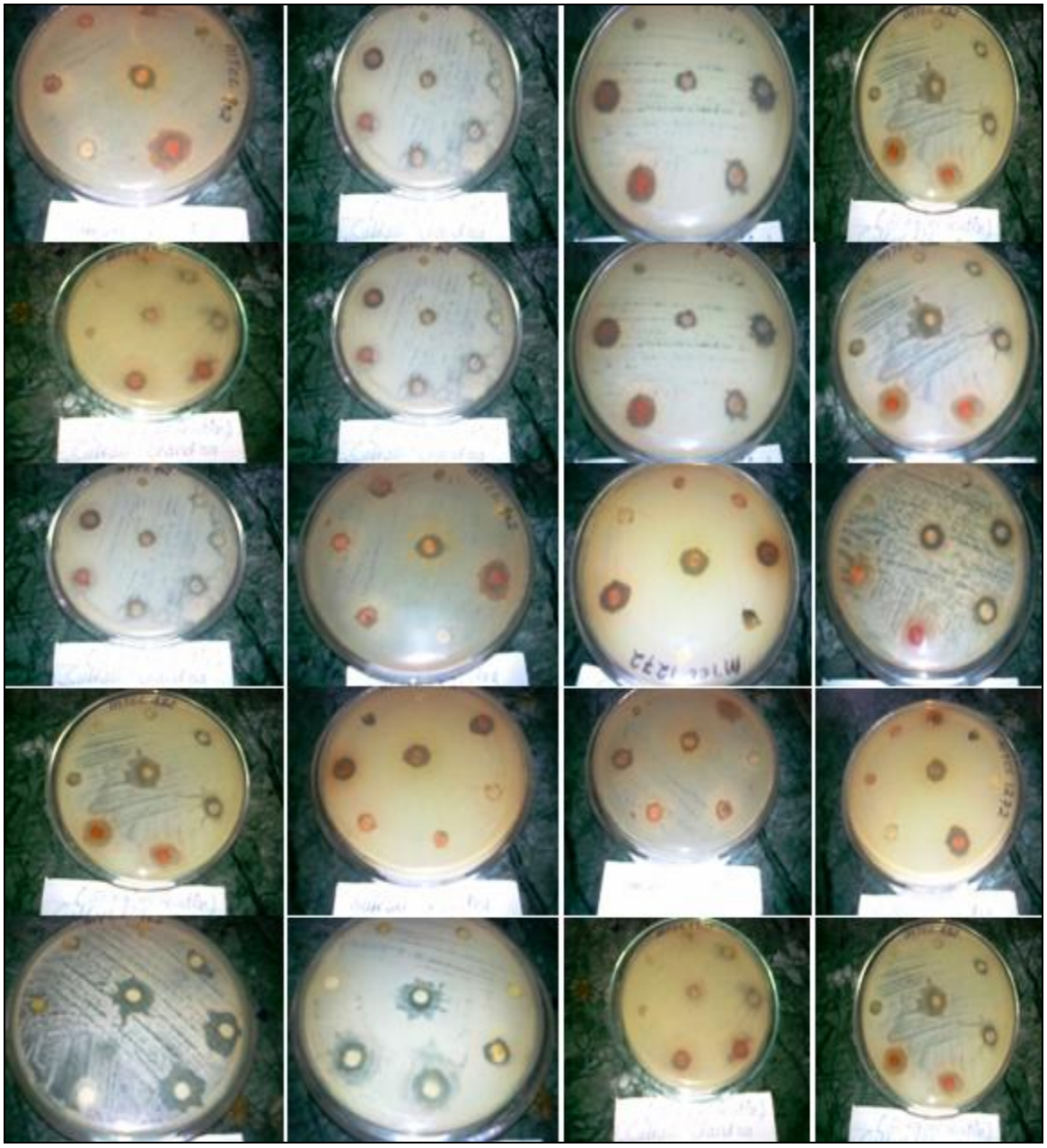

**FIG. 1: ANTIMICROBIAL ACTIVITY OF TEN BACTERIAL STRAINS & THREE FUNGAL STRAINS AGAINST *PYRUS PASHIA* PLANT EXTRACT**

**TABLE 3: NUTRITIONAL VALUE OF *PYRUS PASHIA* FRUIT**

| Nutrients | | Value | |
|---|---|---|---|
| Moisture (%) | 60.36 ± 0.25 | Ascorbic acid | 1.80± 0.05 |
| Ash (%) | 1.10 ± 0.05 | Energy value K Cal | 141.12± 0.12 |
| Total nitrogen (%) | 0.52 ± 0.07 | N (Mg/100gm) | 0.68± 0.06 |
| Total protein (%) | 3.28 ± 0.04 | Ca (Mg/100gm) | 0.75 ± 0.06 |
| Crude fat (%) | 1.62 ± 0.20 | Mg (Mg/100gm) | 0.12± 0.02 |
| Crude fibre (%) | 5.26 ± 0.05 | K (Mg/100gm) | 3.21± 0.05 |
| Carbohydrate | 28.38± 0.12 | P (Mg/100gm) | 0.86 ± 0.04 |
| Organic matter | 98.90± 0.14 | Fe (Mg/100gm) | 0.008 ± 0.02 |

**TABLE 4: PHYTOCHEMICAL SCREENING OF WILD EDIBLE FRUITS P. P – *PYRUS PASHIA***

| Test | PPR | PPB | PPF |
|---|---|---|---|
| **Carbohydrates/glycosides** | | | |
| (1) Molish test | (+) | (+) | (+) |
| (2) Fehling test | (+) | (+) | (+) |
| (3) Benedict test | (+) | (+) | (+) |
| **Alkaloid** | | | |
| (1) Mayer's test | (+) | (+) | (-) |
| (2) Dragendroff test | (-) | (+) | (+) |
| **Flavonoids** | (-) | (+) | (+) |
| **Saponins** | (-) | (+) | (-) |
| **Tannins** | | | |
| (1) Pyrogoll & catechol | (+) | (+) | (-) |
| (2) Gallic acid | (+) | (+) | (-) |
| **Unsaturated sterol/triterpenes** | | | |
| (1) Liebermann Burchard test | (+) | (+) | (-) |
| (2) Salkowski's test | (-) | (+) | (-) |
| Resin | (-) | (+) | (-) |

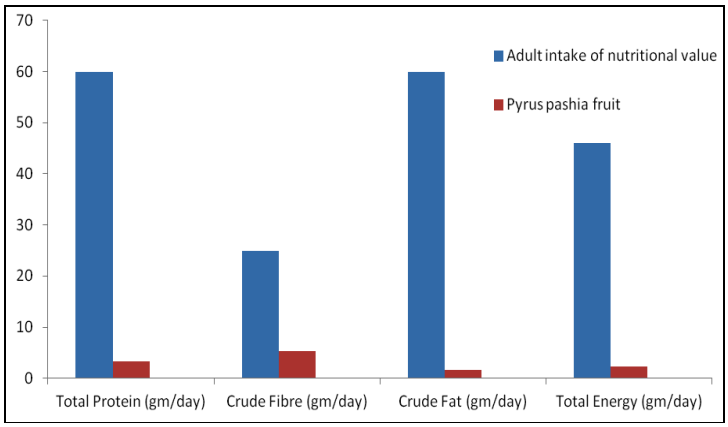


**FIG. 2: COMPARISON OF PER DAY INTAKE OF NUTRIENTS BY ADULTS WITH THE NUTRIENTS PRESENT IN THE FRUITS OF *PYRUS PASHIA***

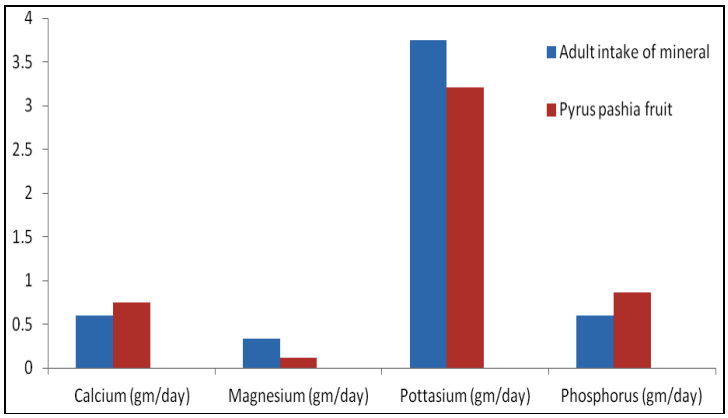


**FIG. 3: COMPARISON OF PER DAY INTAKE OF MINERALS BY ADULTS WITH THE MINERAL PRESENT IN THE FRUITS OF *PYRUS PASHIA***

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## REFERENCES:

1. Srivastava, J. J. Lambert and N. Vietmeyer, 1996. Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320.
2. Balandrin MF, Klocke JA, Wutule ES, Bollinger WH. Natural plant chemicals: Sources of industrial and medicinal materials. Science 1985; 228: 1154– 1160.
3. Satish S, Raveesha KA, Janardhana GR. Antibacterial activity of plant extracts of phytopathogenic *Xanthomonas campestris* pathovars. Lett Appl Microbiol 1999; 28: 145–147.
4. Jones FA. Herbs-useful plants. Their role in history and today. Eur J Gastroenterology Hepatol 1996; 8: 1227–1231.
5. Erdogrul OT (2002). Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biol. 40:269–273.
6. Lin J, Opak War, and Geheeb-Keller M. 1999. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. Journal of Ethnopharmacology, 68: 267–274.
7. Iennette E.H. 1985. Manual of clinical microbiology, 978–987. 4th edition. American Association for Microbiology, Washington.

8. Rosoanaivo and Ratsimanaga Urverg 1993. Biological evaluation of plants with reference to the Malagasy flora. Monograph for the IFs. NAPRECA Workshop on Bioassays.
9. Taylor, R.S.L., N.P. Manandhar, J.B. Hudson and G.H.N. Towers, 1995. Screening of selected medicinal plants of Nepal for antimicrobial activities. J. Ethnopharmacol., 546: 153-159.
10. Espinel Ingroff, A., A. Fothergill, J. Peter, M .G. Rinaldi, and T.J. Walsh. 2002. Testing conditions for determination of minimum fungicidal concentrations of new and established antifungal agents for Aspergillus spp.: NCCLS Collaborative Study. Journal of Clinical Microbiology 40:3204-3208.
11. Gaur, R. D., Flora of the district Garhwal North West Himalaya, 1st Ed. Transmedia Srinagar Garhwal, July-1999,
12. Dutta NK and Iyer SN. Anti-amoebic value of berberine and kurchi alkaloids. Journal of the Indian Medical Association 1968, 50(8), 349-354.
13. Badoni S. Rawat M.S.M., Negi Y.S. Nutritional Composition of some Berberis species, J. Ind. J. of Hort. Pub.-1994
14. Tona, L., Kambu K., Ngimbi N., Cimanga K., and Vlietinck A.J., 1998. Antiamoebic and phytochemical screening of some Congolese medicinal plants. J. Ethnopharmacol., 61: 57- 65.

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