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# ANTIMICROBIAL ACTIVITY OF *PAEDERIA FOETIDA*.L AGAINST ENTEROPATHOGENS

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## ABSTRACT

Ayurvedic medicine *Kolthee churna*, known to be effective in diarrhoea diseases. *Paederia foetida* L. commonly known as the Gandal patta. The antibacterial activities of n-hexane, chloroform, ethyl acetate fractions of methanolic extracts of the whole plants *Paederia foetida* L. (family Rubiaceae) were screened against various pathogenic bacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sp. Vibrio cholereae*, by 'agar cup plate method'. The Ethanol extract of the whole plant possesses antimicrobial activity but the n-hexane fractions exhibited moderate to less activity against some organisms tested compared with the standard antibiotic Metronidazole. The ethanol and the hexane extract showed highest antibacterial activity against ***Salmonella typhi, Vibrio cholerae,*** Moderate antibacterial activity against**,** ***Enterobacter sp, E.coli.*** lowest antibacterial activity against ***Enterococcus sp., Bacillus substilis.***

**KEYWORDS:** *Paederia foetida*, Antimicrobial activity, Methanol extractive *Enterobacter* sp.

## INTRODUCTION

Human is using plants since years to treat various diseases, disorder and as food.[1] The interaction of human and plants lead to the establishment of the traditional knowledge of plants. Through scientific research, human has proved some plants have chemical active compounds with biological functions.[2]

*Paederia foetida* have been reported to have anti-inflammatory, antidiarrheal, antitumor, antimicrobial, anti-tussive and hepatoproctective activities and also used for the treatment of rheumatism and aphrodisiac[3,4] Traditionally the plant may also be used for the treatment of piles, and also for toothache.[5]

*Paederia foetida* is important as medicinal herbs and medical plants since it contains various phytochemical compounds such as Phytochemical investigations reported paederolone, paederone, β-sitosterol, paederoside, asperuloside and their related glucosides.[6] The leaves of the plantare also rich in carotene, vitamin C, keto-alcohol and alkaloid. Shreedhara reported to quantify important markers (asperuloside, beta-sitosterol and lupeol) also in leaves. The aerial parts of the plant contain iridoid glucosides (asperuloside, scandoside and paederoside).[7]

*P. foetida* also contains friedelin, campesterol, ursolic acid, hentriacontane, hentriacontanol, ceryl alcohol, palmitic acid and methyl mercaptan. Ellagic acids Epifriedelinol, Terpenoids, alkaloids paederine (α-paederine and β-paederine), volatile and an essential oil.[7,8]

Content of active chemical compounds in plants are influence by cultivation condition and season of collection, whereby under sunny condition and if the leaves was collected in the summer amount of active compounds are more abundant.[9]

It is usually found in Himalayas from Dehradun eastwards upto an altitude of 1800m and also in Assam, Bihar, Orissa, and Bangladesh. It is a slender, perennial herb.

## MATERIALS AND METHODS

- **Collection of Plant Materials**

The plant materials *Paederia foetida* leaves were collected from local market. Badu, Kolkata in the month of September.

- **Preparation of plant extracts**

The leaves and the stalks were washed, cut into small pieces and then dried under the sun for seven days. The dried leaves and stems then grounded to form a coarse powder with the help of an attrition type of a grinder.[10] The weight of the dried material was 1Kg 250 mg.

- **Ethanolic Extractive preparations**

About 750 gm of powdered leaves was submerged under 5 liters of ethanol in a percolator. The container with its content was sealed and kept for 14 days with occasional shaking and stirring. The mixture was then filtered successively through a piece of clean white cotton. The filtrate thus obtained kept in an open air for the evaporation of the ethanol. After filtration, the extracts were evaporated to dryness to yield the extract.[11] The net weight of the dried extractive yielded 19.2 gms.

- **Hexane Extractive preparations**

About 350 gm of powdered leaves was submerged under 3 liters hexane in a percolator. The container with its content was sealed and kept for 14 days with occasional shaking and stirring. The mixture was then filtered successively through a piece of clean white cotton. The filtrate thus obtained kept in an open air for the evaporation of the hexane. After filtration, the extracts were evaporated to dryness to yield the extract.[11] The net weight of the dried extractive yielded 3.9 gms.

- **Phytochemical screening**

Ethanolic extractive of the plant was quantitatively tested by using standard method for the presence of chemical constituents such as, alkaloids, phenols, flavonoids, saponin, glycosides, steroids and terpenoids.[15,16]

**Table: 1**

| Chemical Constituents | Chemical tests | *Paederia foetida* |
|---|---|---|
| Alkaloid | Mayer's test | + |
| Phenols | Potassium dichomate | + |
| Flavonoid | Shinoda test | + |
| Terpenoid | Salkowski's test | ++ |
| Saponins | Form test | + |
| Steroid | Salkowski's test | ++ |
| Glycosides | Keller-Kilan test | + |

**Test microorganisms**

Test organisms *E.coli, Salmonella typhi, Vibrio cholerae, Bacillus subtilis, Enterococcus sp.* & *Enterobacter sp.* were taken from the staock culture of Institute of Genetic Engineering, Badu, Kolkata.

**Antibacterial assay**

**Preparation of inoculums**

The 20□l of sample microbial culture taken from fresh stock culture and spreaded over the MRS agar(*Bacillus subtilis);* SS agar (*Salmonella typhi),* TCBS agar (*Vibrio cholerae),* Nutrient agar (*Enterococcus sp.),* Enterobacter enrichment agar media*(Enterobacter sp.),* EMB Agar*(E.coli)* plates. Single colony was inoculated in Luria Broth, then incubated for 24hrs at 37˚c. The turbidity of the medium indicates the growth of micro-organism. Then all the cultures were kept at 4˚c until further use.[12]

**Determination of Minimum Bacterial Concentration (MBC)**

The antimicrobial assay was performed by Agar cup plate diffusion method. All collected fractions of the plant like n-hexane, ethyl acetate and chloroform were tested along with the methanol extracts of the whole plants for antimicrobial study by using standard agar cup plate method. In this study, 6 microorganisms which were obtained from the Institute of Genetic Engineering, University of MAKAUT, West Bengal. Standard metronidazole (20 µg/disc) and blank sterile filter paper disc were used as positive and negative controls respectively. Nutrient agar medium was used to prepare fresh cultures for testing the sensitivity of the organisms. Wells were prepared for extractive application and the sample discs, the standard antibiotic discs and the control discs were placed gently on the previously marked zones in the agar plates which were previously inoculated with test bacteria. The discs were then incubated on the plate aerobically at 37ºC for 24 hours. The diameter of zone of inhibition around the cup and disc were measured and recorded at the end of the incubation period to determine the minimum bacterial concentration.[13,14]

**RESULTS AND DISCUSSIONS**

The diameters of the zone of inhibition of the growth were measured by the use of scale ruler in milliliter (mm) clear zones of inhibition indicated the susceptibility of the organism to the extracts while absence of such zones showed resistance or no inhibitory effect of extracts on the test organism.

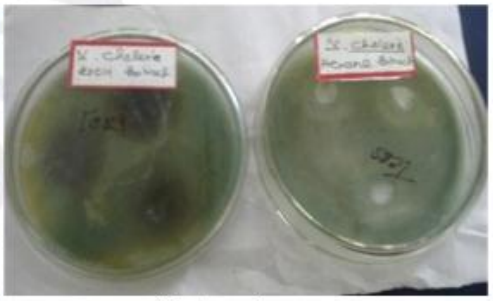
**Table 2: MBC determination of *Paederia foetida* ethanol extract(mg/ml).**

| Organisms(20µl) | Volume of *Paederia foetida* (µl ) | | |
|---|---|---|---|
| | 50 | 100 | 150 |
| | Mean zone of inhibition Diameter (cm) | Mean zone of inhibition Diameter (cm) | Mean zone of inhibition Diameter (cm) |
| ***Salmonella typhi*** | 0.7 | 1.0 | 1.2 |
| ***Vibrio cholerae*** | 0.9 | 1.1 | 1.8 |
| ***Bacillus substilis*** | 0.5 | 0.8 | 0.8 |
| ***Enterococcus sp.*** | 0.5 | 0.7 | 1.1 |
| ***E .coli*** | 0.6 | 0.7 | 0.9 |
| ***Enterobacter sp.*** | 0.6 | 0.9 | 1.2 |

**Table 3: MBC determination of *Paederia foetida Hexane* extract Concentration (mg/ml).**

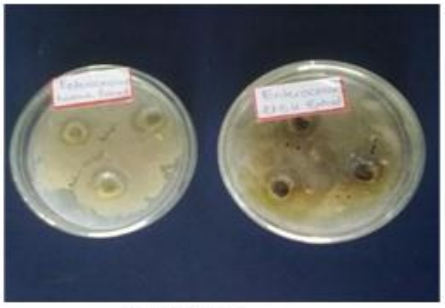
| Organisms(20ml) | Volume of *Paederia foetida* (µl) | | |
|---|---|---|---|
| | 50 | 100 | 150 |
| | Mean zone of inhibition Diameter (cm) | Mean zone of inhibition Diameter (cm) | Mean zone of inhibition Diameter (cm) |
| ***Salmonella typhi*** | 0.8 | 1.2 | 1.3 |
| ***Vibrio cholerae*** | 0.8 | 0.9 | 1.2 |
| ***Bacillus substilis*** | 0.0 | 0.0 | 0.5 |
| ***Enterococcus sp.*** | 0.6 | 1.0 | 1,3 |
| ***E .coli*** | 0.7 | 0.7 | 0.9 |
| ***Enterobacter sp.*** | 0.5 | 0.6 | 0.9 |

Picture 1

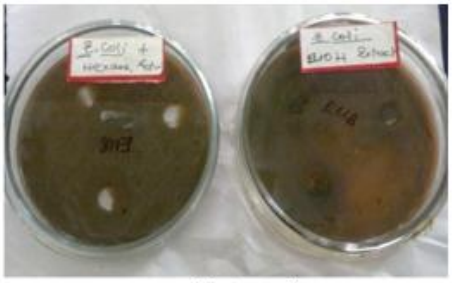


Picture 2

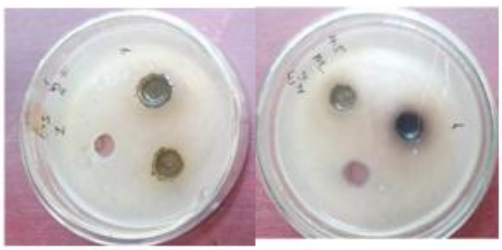
Picture 3



Picture 4



Picture 5



Picture 6

**Table 4: Comparison of the extractive with a broad spectrum antibiotic.**

| Organism(20µl) | Antibiotic metronidazole Conc. (1.0%) Positive control | | | Sterile distilled water Negative control | | |
|---|---|---|---|---|---|---|
| | 50(µl) | 100(µl) | 150(µl) | 100(µl) | 200(µl) | 300(µl) |
| | Mean zone Zone of inhibition (cm) | | | Mean zone of inhibition Diameter (cm) | | |
| ***Salmonella typhi*** | 0.9 | 0.9 | 1.1 | 0.0 | 0.0 | 0.0 |
| ***Vibrio cholera*** | 1.5 | 1.6 | 1.5 | 0.0 | 0.6 | 0.0 |
| ***Bacillus substilis*** | 1.1 | 1.1 | 1.1 | 0.0 | 0.0 | 0.0 |
| ***Enterococcus sp.*** | 0.5 | 0.5 | 0.9 | 0.0 | 0.0 | 0.0 |
| ***E.coli*** | 1.0 | 1.0 | 1.0 | 0.0 | 0.0 | 0.0 |
| ***Enterobacter sp.*** | 0.0 | 0.0 | 0.6 | 0.0 | 0.0 | 0.0 |

**Determination of MIC**

All pathogens were selected for MIC studies for both the extractives because of their potential inhibitory effect against the plants extracts when compared to the other microbial organisms. The extract concentration for MIC of the ethanolic and hexane extractives of *Paederia foetida* was made as 1mg /ml. According to the MBC results the concentrations 30, 35, 40, 45 and 50 mg/ml were used to determine the MIC. The MIC was recorded as the least concentrations of the extract that completely inhibited the growth of the test organisms about 20µl of culture added on 5ml of Luria broth and incubated 24hrs to determined antimicrobial activity. Antimicrobial activity was determined when no growth occurred on the sub-culture medium after MIC determination.[13,14]

**Table 5: Minimum inhibition of concentration ethanol extract(mg/ml) (MIC).**

| Organisms | Extractive in µl | | | | |
|---|---|---|---|---|---|
| | 30 | 35 | 40 | 45 | 50 |
| **1. *Salmonella typhi*** | + | + | - | - | - |
| **2. *Vibrio cholera*** | + | - | - | - | - |
| **3. *Bacillus substilis*** | + | + | + | + | - |
| **4. *Enterococcus sp.*** | + | + | + | + | - |
| **5. *E.coli*** | + | + | + | - | - |
| **6. *Enterobacter sp.*** | + | + | + | - | - |

+ sign denotes the presence of growth and negative sign is for absence of growth of the microorganism at the respective concentration of the extractive.

- **Table 6: Minimum inhibition of concentration hexane extract((mg/ml) (MIC).**

| Organisms | Extractive in µl | | | | |
|---|---|---|---|---|---|
| | 30 | 35 | 40 | 45 | 50 |
| **1. *Salmonella typhi*** | + | + | - | - | - |
| **2. *Vibrio cholera*** | + | + | - | - | - |
| **3. *Bacillus substilis*** | + | + | + | + | + |
| **4. *Enterococcus sp.*** | + | + | + | - | - |
| **5. *E.coli*** | + | + | + | + | - |
| **6. *Enterobacter sp.*** | + | + | + | + | - |

+ sign denotes the presence of growth and negative sign is for absence of growth of the microorganism at the respective concentration of the extractive.

**DETERMINATION OF MICROBIOCIDAL OR MICROBIOSTATIC ACTIVITY**

Loopful culture from the negative growth test tube after 24 hours incubation were reinoculated in 5 ml fresh culture media and incubated again without adding the extract for 24 hours.

Turbidity or growth in the fresh media was observed. Therefore, the extractive at this concentration is acting as MICROBIOSTATIC agent.

The tested plant showed the antimicrobial activity which have been represented in table 2&3. Considering *Peaderia foetida* ethanol extract showed the better inhibition than hexane extracts.

Phytochemical studies reveal that alkaloids, phenols, flavonoids, saponin, glycosides, steroids and terpenoids are present in this selected plant.(Table:1).

The secondary metabolites includes alkaloids, saponins, terpens possess antibacterial actions.[17]

Experimental observations reveals that both the ethanol and hexane extractive possessed antibacterial activity against enteropathogens (Table: 2,3).

The extractive is very much effective against *Salmonella typhi and Vibrio choleriae*. The minimum inhibitory concentrations for the *P. foetida* ethnol extractive against *Salmonella typhi* was 35 to 40 µl. and against *Vibrio choleriae* was 30 to 35 µl. The minimum inhibitory concentrations for the *P. foetida* hexane extractive against *Salmonella typhi* was 35 to 35 µl. and against *Vibrio choleriae* was 35 to 35 µl. (picture 1,2).

The extractive is moderately effective against *Enterococcus* sp. *and Bacillus subtilis*. The minimum inhibitory concentrations for the *P. foetida* ethnol extractive against *Enterococcus* sp. was 45 to 50 µl. and the hexane extractive against *Enterobacter sp* was 40 to 45 µl. The minimum inhibitory concentrations for the *P. foetida* ethanol extractive against *Bacillus subtilis* was 45 to 50 µl. and hexane extractive against *Bacillus subtilis* was above 50 µl. (picture 3,4).

The extractive is also effective against *E.coli and Enterobacter sp.* The minimum inhibitory concentrations for the *P. foetida* ethnol extractive against *E.coli* was 40 to 45 µl. and against *Enterobacter sp* was 40 to 45 µl. The minimum inhibitory concentrations for the *P. foetida* hexane extractive against *E.coli* was 45 to 50 µl. and against *Enterobacter sp* was 45 to 50 µl. (picture 5,6).

## CONCLUSION

In this qualitative analysis, it was proved that potent active antimicrobial principles are present in the *Paederia foetida* L. leaf ethanolic extractive as well as hexane extractive. Extractives were extensively inhibited the growth of test organisms *Salmonella typhi*, *Vibrio cholera*, *E.coli*, *Enterobacter sp.* and moderately of *Bacillus subtilis* and *Enterococcus sp.*

The anti diarrhoeagenic activity of *Paederia foetida* extract is quiet comparable to mertronidazole.

According to the spectrum of anti diarrhoeagenic activity of *Paederia foetida* extract, it can be stated that the plant extractive or the active principle of it may be the alternative of the antibiotics used for enteric disorders.

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**Competing Interests Statement**

The authors declare that they have no competing interests.

**Data Sharing Statement**

We cannot share any unpublished data with other laboratory or person.

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