



**International Journal of Biology, Pharmacy
and Allied Sciences (IJBPAS)**
'A Bridge Between Laboratory and Reader'

www.jibpas.com

**PHYTOCHEMICAL STUDIES ON TRADITIONAL MEDICINAL PLANTS OF
NEPAL AND THEIR FORMULATIONS**

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ABSTRACT

Nepalese medicinal plants *Hedychium spicatum* Buch.-Ham., *Astilbe rivularis* Buch.-Ham. ex D. Don, *Lycopodium clavatum* L., both leaf and bark of *Rhododendron arborium* Smith, Exot. Bot., *Piris Formosa* (Wallich) D. Don, *Achyranthus aspera* L., *Gaultheria fragrantissima* Wall., *Smilax aspera* L., *Calotropis gigantia* R. Br., *Cuphea procumbens* Ort. Perrito, *Oroxylum indicum* (Linn.) Vent, and *Parmelia spp* were extracted in methanol and used for phytochemical test and biological activities. Plants were found rich in alkaloids, coumarins, tannins, sterols, triterpenes, saponins, glycoside and flavonoids. Cytotoxicity test showed that, plants, *Astilbe rivularis* Buch.-Ham. ex D. Don, *Gaultheria fragrantissima* Wall., *Cuphea procumbens* Ort. Perrito, *Oroxylum indicum* (Linn.) are non toxic. Phytochemical screening of extracts obtained from *Hedychium spicatum* Buch.- Ham, *Lycopodium clavatum* L., both leaf and bark of *Rhododendron arborium* Smith, Exot. Bot., *Piris Formosa* (Wallich) D. Don, *Achyranthus aspera* L., *Calotropis gigantia* R. Br., *Parmelia spp*, showed the presence of alkaloids in majority of plants except *Smilax aspera* L. Antimicrobial susceptibility test showed that *Astilbe rivularis* Buch.-Ham. ex D. Don has shown the prominent results against the *Kliebsiella pneumonie*. Formulation of ointment was successfully prepared and found positive for its various applications.

**Keywords: Nepalese Medicinal Plants, Phytochemistry, Cytotoxicity, Antimicrobial,
Ointment**

INTRODUCTION

Medicinal plants are used for primary health care not just in rural areas in developing countries, but also in developed countries as well where modern medicines are predominantly used. Natural products have been the single most productive source and these have played a vital role in the discovery of new chemical entities of drug discovery as a primary source of commercial medicines and drug leads. The central Himalaya is a huge repository of such medicinal plants. Due to species climatic and geographical conditions, temperate and alpine plants of the Himalaya offer greater possibilities of having novel molecules and even largest quantities of the active compounds. Nepal for being located at this portion of the Himalaya, has always remained a place of great interest to the botanists and phytochemists involved in researching medicinal herbs. Medicinal plants are an important component of the vegetation of Nepal. Despite many studies on medicinal plant resources of Nepal, a large number of medicinal plants and associated indigenous uses still wait proper documentation and evaluation of their therapeutic properties. Many studies have investigated traditional uses of such plants, but only a few studies have followed these ethnobotanical investigations with laboratory work to verify the actual

therapeutic property of these plants [1-5]. In Nepal, more people seek medical attention from traditional medical practitioners than from modern allopathic practitioners. Medicinal plants especially serve as the main source of medicine to rural poor communities that do not have access to modern medical services. Many such practices were experimentally proved depicting the scientific insight behind their traditional adoption. This attempts to prove scientific insight behind the traditional adaption. Less toxicity, better therapeutic effect, good patient compliance and cost effectiveness are the reasons for choosing drug from natural origin.

A herbal extract is a concentrated solution made from extracting constituents out of the structural matrix of an herb. Such extract has the typical property of a viscous plant extract and other properties associated with the powdered herbs and plants. Along with other dosage forms, herbal drugs are also formulated in the form of tablets, syrups, ointments etc. . Medicated herbal ointments contain a medicament dissolved, suspended or emulsified in the base. Ointment bases are almost always anhydrous and generally contain one or more medicaments in suspension or solution or dispersion. Ointment bases may be hydrocarbon, absorption, water removable and water

soluble type. This study was aimed to demonstrate the feasibility and application of modern drug formulation techniques in traditional medicines.

MATERIALS AND METHODS

Collection of the Plant Material

The collection of the plants was done from May-June 2012. One of the collection site is located in the Sapang village of Kavrepalanchok District in Bagmati zone in the Central Development Region of Nepal. The site is located between the latitude and longitude of 27.66° N and 85.75°E respectively. Another collection site is Batase village of Kavrepalanchok District in Bagmati zone in Central Development Region of Nepal. This site is located between the latitude and longitude of 27.81°N and 85.74°E respectively. The collected plant samples making herbarium were kept in Department of Pharmacy, Kathmandu University and plants were identified by Rajendra Gyawali and Tirtha Maiya Shrestha, teaching faculty of the Department of Pharmacy, Kathmandu University, Nepal. The collected plant materials of *Hedychium spicatum* Buch.-Ham., *Astilbe rivularis* Buch.-Ham. ex D. Don, *Lycopodium clavatum* L., both leaf and bark of *Rhododendron arborium* Smith, Exot. Bot., *Piris Formosa* (Wallich) D. Don, *Achyranthus aspera* L., *Gaultheria fragrantissima* Wall., *Smilax aspera* L,

Calotropis gigantia R. Br., *Cuphea procumbens* Ort. Perrito, *Oroxylum indicum* (Linn.) Vent, and *Parmelia spp* were then dried in shade and stored at room temperature before the experiments.

Preparation of Extracts

Cold extraction was carried out using methanol. The whole dried plant sample was blended in home blender and powdered sample was initially soaked in methanol in a conical flask. The mouth of the flask was closed with aluminum foil to reduce the volatilization of the solvent. The flask was allowed to stand for 7 days with occasional shaking. After 7 days, the solvent along with solubilised components were collected. The remaining extracted residue was re-extracted by adding fresh methanol. The extraction process was carried out three times with the same sample. Thus total extraction duration altogether was 21 days. The individual liquors were in vacuum and the dried extracts weighed to obtain the extract and yield was calculated in each case.

Phytochemical Test

All the extracts of plants were screened for the presence of alkaloids, glycosides, tannins, saponins and flavonoids according to standard procedure [6].

Cytotoxicity Assay

Extracts of plants were evaluated for lethality to brine shrimp larvae (*A. salina*

Leach). Brine shrimp eggs were hatched for 48 h in a conical flask containing 300 mL of artificial seawater. The flasks were well aerated with the aid of an air pump and kept in a water bath at 29–30 °C. The extracts were dissolved in 1 % aqueous DMSO and then in sea water to obtain a concentration of 800 ppm, 400 ppm, 200 ppm, 100 ppm, 50 ppm, 25 ppm and 12.5 ppm. An aliquot of each concentration (1 mL) was transferred, in triplicate, into clean sterile vials with pipette, and aerated seawater (9 mL) was added. Ten shrimp nauplii were transferred to each vial. Thymol 1% aqueous solution and 1% DMSO in seawater were used as positive and negative controls, respectively. After 24 h the numbers of survivors were counted and percentage of death calculated. The concentration that killed 50 % of the nauplii (LC50 in µg/mL) was determined. Criterion of toxicity for fractions was established according to Déciga-campos et al. 2007: LC50 values > 1000 µg/mL (non-toxic), $500 \leq 1000$ µg/mL (weak toxicity) and < 500 µg/mL (toxic).

Antimicrobial Assays

Antimicrobial properties has been carried out by accoding to standard guidelines [7]. In this study, human pathogenic bacterial strains, *Kleibsella Pneumoniae*, *Salmonella Typhii*, *Pseudomonas sps.*, and *Staphylococcus aureus* collected from

Dhulikhel hospital, Kathmandu University teaching hospital, were used to investigate the antimicrobial potential of the extracts by disc diffusion method. The microorganisms were kept under refrigeration (4 °C) until use. Stock solutions of petroleum ether, ethyl acetate, and methanolic extracts were prepared in 1 % aqueous DMSO and then in RO water. The solution was then diluted to give final concentrations ranging from 4, 6 and 8 % w/v and nitrofurantoin discs were used as positive controls 13. The microorganism cultures were grown in Muller Hinton agar media. Each micro-organism, at a concentration of 1.5×10^6 cells/mL (adjusted to the 0.5 McFarland turbidity standards) was inoculated on the surface of respective media. Diluted extracts were used to prepare 6 mm diameter disk by dipping them in solutions. After holding the plates at room temperature for 1 h to allow diffusion of test samples into the agar, they were incubated at 37°C for 24 hr. After that, the results were recorded by measuring the zones of growth inhibition around the discs, and presented as the arithmetic average. Overall, cultured microorganisms with halos equal to or greater than 7 mm were considered susceptible to samples tested.

Formulation of Ointment

Oil was extracted from plant *Gultheria fragrantissima* with the help of Clevenger's

apparatus. The extracted oil was used as an active ingredient in the ointment. The ointment was formulated by using methyl salicylate, bee wax and hydrous wool fat, according to the formula as given in BP 2007 [8].

Formulation of *A. ribularis* Tablet

The extract obtained from *A. ribularis* was prepared to manufacture the tablet each of 300 mg. Direct compression was done for the tablet. Extract was dissolved in Iso Propyl Alcohol. The dissolved solution was dissolved in Aerosil and was sieved through 80 mesh. The mixture was added with MCC in geometric fashion and was shaken for 10 minutes. PVK 30 was added and mixed for 10 minute and then Magnesium Stereate was added and mixed for 2 inutes. The tablet was then compressed using 10 mm die. Master formula of *A. ribularis* formulation was Extract (1.1mg), Aerosil (23mg), PVK30 (3mg), Magnesium Stearate (3mg), MCC (269.1mg) for 300 mg tablet.

Physiochemical Evaluation

Hardness Test: It is the load required to crush the tablet when placed on its edge. It was done with the help of Monsanto hardness tester.

Friability: It is the tendency of tablets to powder, chip, or fragment and this can affect the elegance appearance, consumer acceptance of the tablet, and also add to tablet's weight variation or content

uniformity problem. This test was done according to USP NF32 [9].

Disintegration Test: It is the time required for the tablet to break into particles, the disintegration test is a measure only of the time required under a given set of conditions for a group of tablets to disintegrate into particles as per USP NF32.

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical screening of extracts obtained from *Hedychium spicatum* Buch.-Ham., *Astilbe rivularis* Buch.-Ham. ex D. Don, *Lycopodium clavatum* L., both leaf and bark of *Rhododendron arborium* Smith, Exot. Bot., *Piris Formosa* (Wallich) D. Don, *Achyranthus aspera* L., *Gaultheria fragrantissima* Wall., *Calotropis gigantia* R. Br., *Parmelia spp*, showed the presence of alkaloids in majority of plants except *Cuphea procumbens* Ort. Perrito, *Smilax aspera* L, *Oroxylum indicum* (Linn.) Vent. Alkaloids posses various pharmacological properties like analgesic, antamoebic, emetic, anticholinergic, antihypertensive, antitumor, skeletal and smooth muscle relaxants. Coumerons, a phenolic compounds were not detected in the species *Astilbe rivularis* Buch.-Ham. ex D. Don, and *Parmelia spp*. Sterols were also detected in *Lycopodium clavatum* L., *Piris Formosa* (Wallich) D. Don,. *Astilbe rivularis* Buch.-Ham. ex D. Don, and *Parmelia spp* . Pharmacological activity of sterol is that it

lowers serum concentration of cholesterol by reducing its absorption from gut. Terpenes are also detected in *Oroxylum indicum* (Linn.) Vent and *Astilbe rivularis* Buch.-Ham. ex D. Don. Terpenes are commonly used in the treatment of acute or chronic bronchitis and related conditions. Both the sterols and terpenes are present in *Rhododendron arborium* Smith, Exot. Bot. (Leaf), *Gaultheria fragrantissima* Wall., *Calotropis gigantia* R. Br., *Astilbe rivularis* Buch.-Ham. ex D. Don.,. Pharmacological activity of sterols and terpenes is that it lowers serum concentration of cholesterol by reducing its absorption from gut. Pharmacological activities of Tannins and Polyphenols is that it acts as Antidiarrheal, Haemostatic, Antihemorrhoidal, Anti-inflammatory, Antiviral, Antibacterial and Antiparasitic. Phytochemical evaluation showed the presence of reducing sugar in *Lycopodium clavatum* L., *Astilbe rivularis* Buch.-Ham. ex D. Don, *Rhododendron arborium* Smith, Exot. Bot. (Bark), *Piris Formosa* (Wallich) D. Don, *Parmelia* spp, *Cuphea procumbens* Ort. Perrito. Reducing sugar forms an aldehyde or ketone in basic form. All the monosachharides containg ketone groups are known as ketoses and those containing aldehydes are known as aldolase. Glycosides were not detected in *Cuphea procumbens* Ort. Perrito, *Oroxylum indicum*

(Linn.) Vent, *Rhododendron arborium* Smith, Exot. Bot. (Leaf). Cardiac glycosides are used in cardiac failure treatment as these have anti arrhythmic property. These prolong the plateau phase of cardiac depolarization thus slowing ventricular contraction. This causes increase in force of contraction thereby increasing cardiac output. Phytochemical evaluation showed the presence of Saponins in *Oroxylum indicum* (Linn.) Vent, *Rhododendron arborium* Smith, Exot. Bot. (Leaf). *Astilbe rivularis* Buch.-Ham. ex D. Don. Pharmacological activity of saponin is that it acts as Antioxidant, Anticancer, Anti-inflammatory, Antiviral, Immune system booster. Phytochemical evaluation showed the presence of Flavonoids in *Rhododendron arborium* Smith, Exot. Bot. (Leaf). *Astilbe rivularis* Buch.-Ham. ex D. Don., *Calotropis gigantia* R. Br., *Piris Formosa* (Wallich) D. Don, *Cuphea procumbens* Ort. Perrito. Pharmacological activities of Flavonoids that it acts as Anti-inflammatory, Antihepatotoxic, Antiulcerative, Antioxidants, Free radical scavengers and maintains membrane integrity.

Determination of Yield Percentage of Various Extracts

The yield value of methanolic extrats of various plants was obtained. *Cuphea procumbens* Ort. Perrito has minimum yield

percent i.e 2% and *Lycopodium clavatum* L. has maximum yield percent i.e 17%. Yield value quantifies the amount of active constituents relative to amount of the crude drug material. Due to unavailability of standard yield values data, we can only assume that such lower yield value may be due to either the plants contains lower amount of active constituents or relatively more number of active constituents are not solubilized in methanol. Extraction yield can be increased by using a different polarity of solvent for solubilizing the compounds towards leading to increased yield.

Brine shrimp Lethality Test

LC₅₀ of *Astilbe rivularis* Buch.-Ham. ex D. Don, *Cuphea procumbens* Ort. Perrito, *Gaultheria fragrantissima* Wall., *Oroxylum indicum* (Linn.) Vent., *Piris Formosa* (Wallich) D. Don, and *Parmelia spp*, is greater than 100µg/ml so these plants are considered as not toxic. LC₅₀ of *Rhododendron arborium* Smith, Exot. Bot., and *Achyranthus aspera* L., is less than 100µg/ml but greater than 30µg/ml so these plants are mild toxic. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted on the graph paper and the values of LC₅₀ are calculated.

Antimicrobial Activity Test

Plants *Hedychium spicatum* Buch.- Ham., *Lycopodium clavatum* L. *Piris Formosa* (Wallich) D. Don, *Smilax aspera* L., *Oroxylum indicum* (Linn.) , *Parmelia spp*, *Cuphea procumbens* Ort. Perrito, *Oroxylum indicum* (Linn.) Vent were shown effective against the *Pseudomonas*. All of the tested plants were not much effective against *E. coli*, except *Astilbe rivularis* Buch.-Ham. ex D. Don. All the plants were very effective against *Salmonella typhi* except *Hedychium spicatum* Buch.- Ham. Plant *Oroxylum indicum* (Linn.) was very much effective against the *Staphylococcus*. *Astilbe rivularis* Buch.-Ham. ex D. Don has also shown the prominent results against the *Kliebsiella pneumonie*.

A very remarkable result we observed during our work is on *Hedychium spicatum* Buch.- Ham., that was found to be microbiologically effective against *E. coli* where ciprofloxacin is resistant and was found to be active against *Salmonella typhi* and *Staphylococcus*. Phytochemical screening of extracts obtained from *Hedychium spicatum* Buch.- Ham., *Astilbe rivularis* Buch.-Ham. ex D. Don, *Lycopodium clavatum* L., both leaf and bark of *Rhododendron arborium* Smith, Exot. Bot., *Piris Formosa* (Wallich) D. Don, *Achyranthus aspera* L., *Gaultheria fragrantissima* Wall., *Calotropis gigantea* R.

Br., *Parmelia spp*, showed the presence of alkaloids in majority of plants except *Cuphea procumbens* Ort. Perrito, *Smilax aspera* L, *Oroxylum indicum* (Linn.). *Achyranthus aspera* L. was found to be active against *Pseudomonas* and *Kliebsella pneumoniae* and also in *e.coli* in 6% concentration. *Gaultheria fragrantissima* Wall. was found to be active against *Pseudomonas* in all three concentrations and in *Salmonella* and *Staphylococcus* in 6% concentration. *Cuphea procumbens* Ort. Perrito was found to be active against *E.coli* and *Pseudomonas* in 4% and 6% concentration but in 6% for *Salmonella typhi*.

A lichen, *Parmelia spp* was found active against *Staphylococcus* and *Pseudomonas* in all three concentrations but for *E.coli* and *Salmonella typhi* in 6%. Also it was found to be active against *Pseudomonas* in 4% concentration. Among all the methanolic extract of lichen spp showed higher antimicrobial activity in all species except in *Kliebsiella pnneumonie* where it has no activity.

Ointment of *Gualtheria fragrantissima*

Irritancy: Irritancy test was done by applying the ointment on the normal skin of healthy volunteers and observing the reaction of the 10 individuals. Result showed that one of them had irritancy but others did not had negative reaction.

Pain subside: When test was done to football players of KUBH, their feedback was positive. They assured of subsiding of pain on twice application.

Spreadability: This test was done by applying the ointment on the skin and noticing whether spreading was good or not. the spreading was good. **Stability test:** Stability test for the final product was carried out by storing the ointment at temperature of -4 °C for 24 hrs. The product remained stable during study period.

Formulation of Ointment

Oil was extracted from plant *Gultheria fragrantissima* with the help of *Clevenger's apparatus*. The extracted oil weighed about 67 gm. The extracted oil was used as an active ingredient in the ointment. The identification test of obtained oil was carried according to BP and all the test results were positive. The oil was slightly yellow coloured, very slightly soluble in water, and miscible with alcohol. 0.25 ml of oil was heated with 2 ml of dilute sodium hydroxide solution on a water-bath for 5 min. Dilute sulphuric acid was added. A crystalline precipitate is formed. It was then filtered. The precipitate, washed with water and dried at 100 °C melted at 158°C which lied in the standard range of 156° C to 161° C. Similarly, 2 ml of the oil was added with 10 ml of alcohol. The solution is clear and not

more intensely coloured than reference solution.

Quality Control Test

Irritancy test was done by applying the ointment on the normal skin and observing the reaction of the 10 individuals. Only one of them had irritancy but others have no reaction. Pain relieving test was done to football players of KUBH, their feedback was positive. They assured of subsiding of pain on twice application. Similarly Spreadability test was done by applying the ointment on the skin and noticing whether spreading was good or not, the spreading was good. Stability test for the final product was carried out by storing the ointment at temperature of -4 °C for 24 hrs. The product remained stable.

Formulation of *A. ribularis* Tablet

The extract obtained from *A. ribularis* was prepared to manufacture the tablet each of 300 mg. Direct compression was done for the tablet. The tablets produced were subjected to test for hardness, friability and disintegration test. The hardness of tablet was found to be around 4.1-5.2. The loss in weight of the tablets was found to be 0.23% which satisfies the result of friability test which states “ *It must be less than or equal to 1%*”. Hence the friability test was considered as pass for this formulation. All tablets disintegrated in 3 min 54 sec which resulted passing of the tablets in

disintegration test according to USP 30 NF 25 [9].

CONCLUSION

It is observed that, the collected plants rich in alkaloids, coumarins, tannins, sterols, triterpenes, saponins, glycoside and flavonoids. Cytotoxicity test showed that, plants., *Astilbe rivularis* Buch.-Ham. ex D. Don, *Gaultheria fragrantissima* Wall., *Cuphea procumbens* Ort. Perrito, *Oroxylum indicum* (Linn.) are non toxic. Phytochemical screening of extracts obtained from *Hedychium spicatum* Buch.-Ham, *Lycopodium clavatum* L., both leaf and bark of *Rhododendron arborium* Smith, Exot. Bot., *Piris Formosa* (Wallich) D.Don, *Achyranthus aspera* L., *Calotropis gigantea* R. Br., *Parmelia spp*, showed the presence of alkaloids in majority of plants except, *Smilax aspera* L. Antimicrobial susceptibility test showed that *Astilbe rivularis* Buch.-Ham. ex D. Don has shown the prominent results against the *Kliebsiella pneumonie*. Formulation of ointment was successfully prepared and found positive for its various applications.

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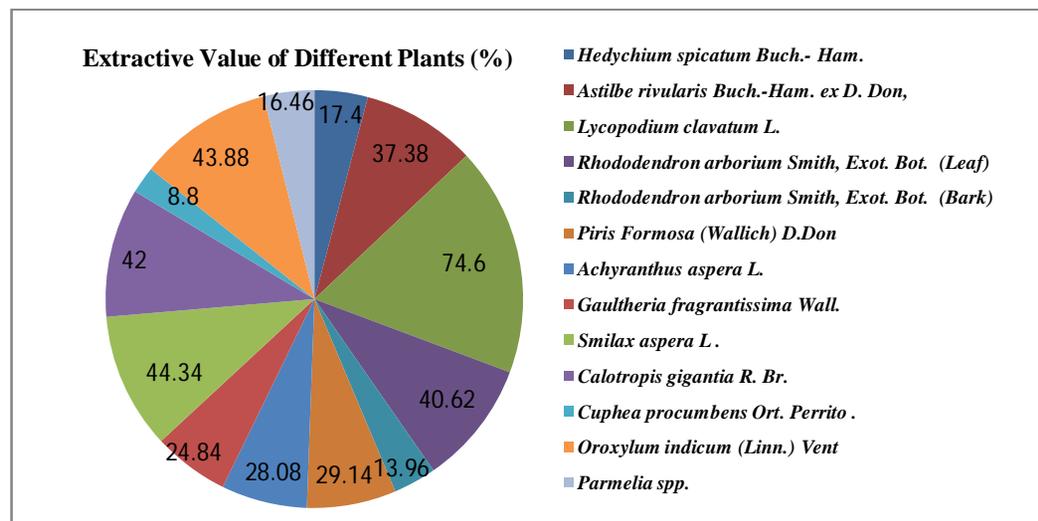


Figure 1: Comparison of Yeild Percentage of Methnolic Extract of Different Plant Under Study

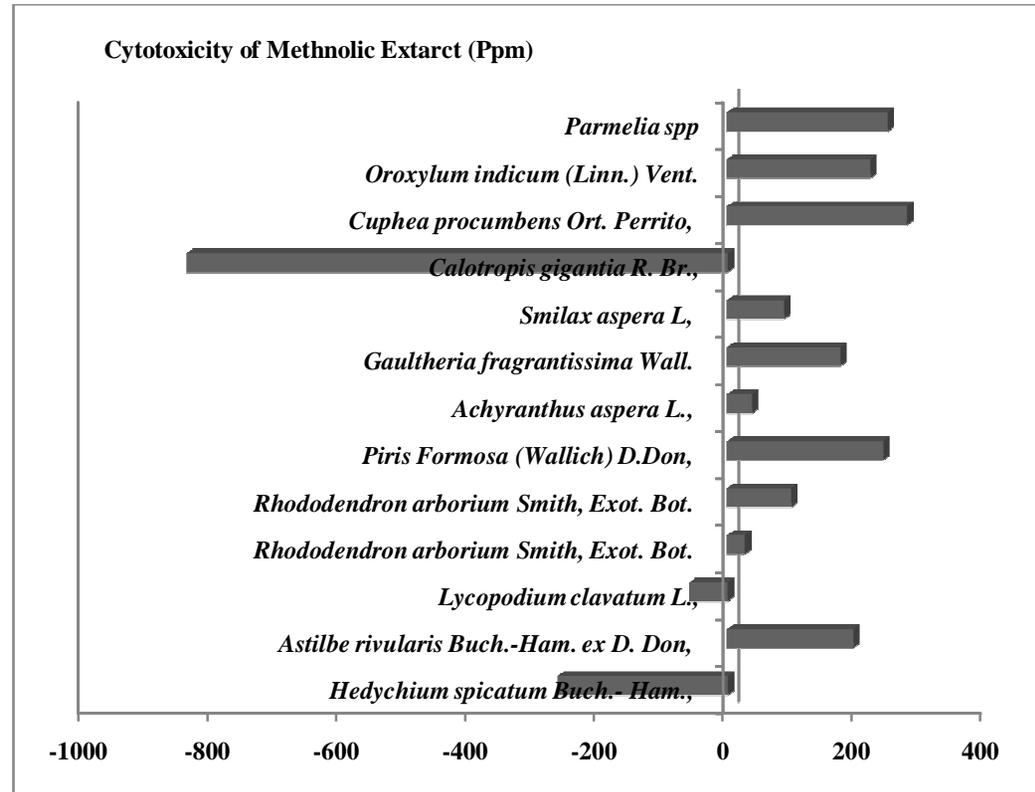


Figure 2 : Comparson of Mortality of Brine Shrimp of Methnolic Extract of Different Plants

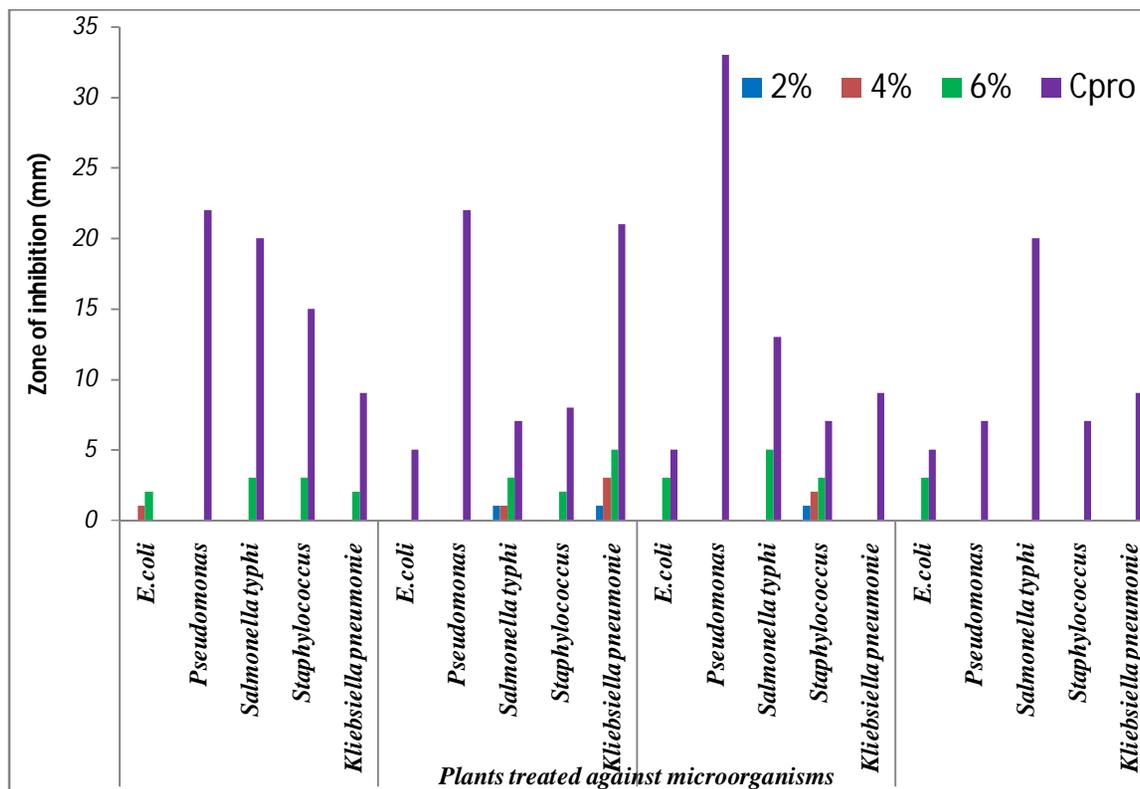


Figure 3: Comparison of Antimicrobial Activity Shown by the Methanolic Extract of *Hedychium spicatum*, *Lycopodium clavatum* and *Rhododendron aborium* (Leaves and Barks)

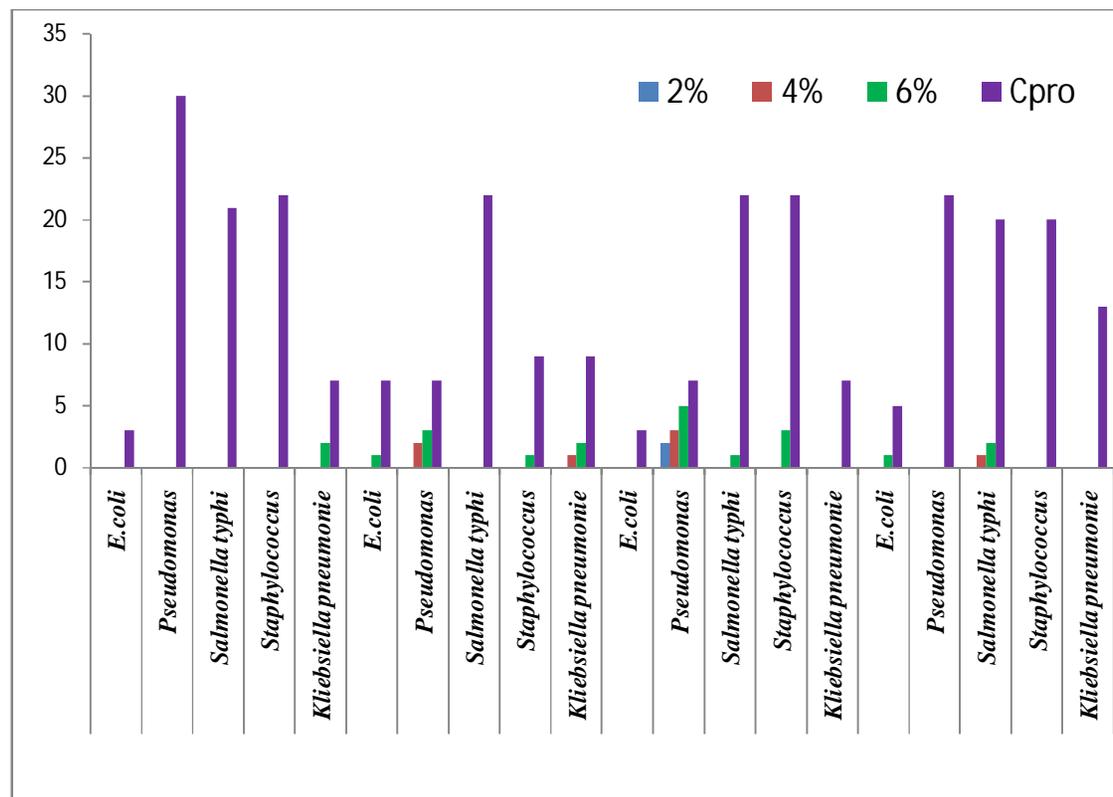


Figure 4: Comparison of Antimicrobial Activity Shown by the Methanolic Extract of *Piris formosa* , *Achyranthus aspera* and *Gualtheria fragrantissima*, *Smilax aspera* L.

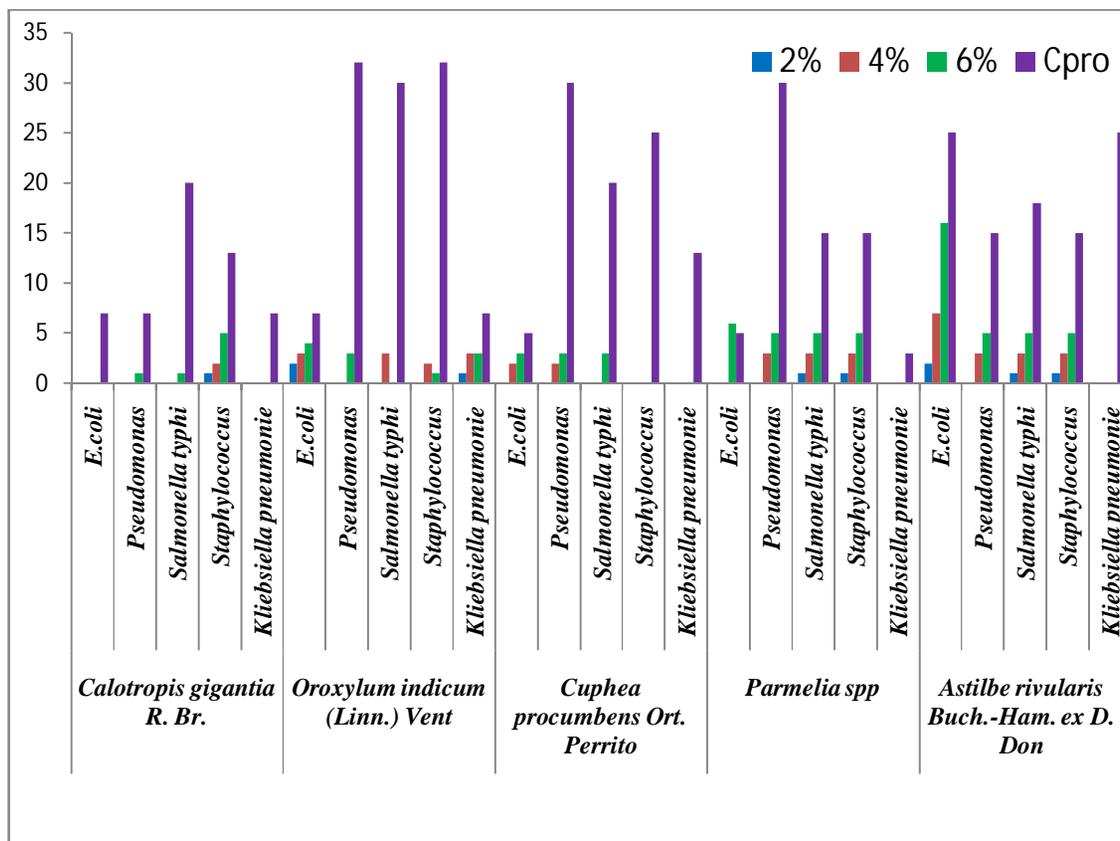


Figure 5: Comparison of Antimicrobial Activity Shown by the Methanolic Extract of *Calotropis gigantea*, *Oroxylum indicum*, *Cuphea procumbens*, *Parmelia* spp. and *Astilbe rivularis*