ISSN: 2320-9267

Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

ORIGINAL ARTICLE

In Vitro Anti Microbial Activity of *Roylea elegans*, *Chrozophora tinctoria*, and *Daphniphyllum himalaense*

Jyoti Gupta[1]*, Ruchika Garg[2], Sanjeev Mittal[1]

ABSTRACT

Herbal combination plays a major role in enhancing the activity due to its synergetic effects. Many well-known plants possess their antibacterial activity individually, and a combination of these may lead to the better efficacy of results. Plants belonging to the family Euphorbiaceae and Laminaceae possess an excellent antibacterial effect against various bacteria. However, secondary metabolites like; terpenes, alkaloids, and phenolic compounds are well for their free radical scavenging activity, which may inhibit the growth of microorganisms. Thus, the combination of these herbal plants is evaluated for antibacterial activity using hydrogel forms solution.

Keywords: Bacteria, Enhancing, Microorganism, Scavenging.

Indian J. Pharm. Biol. Res. (2021): https://doi.org/10.30750/ijpbr.9.3.1

INTRODUCTION

The interest in natural products as a potent therapeutic agent has increased tremendously toward herbal medicine. WHO estimated about 65-80% of the total world population uses traditional medicine as a primary source of healthcare. About 85% of conventional medicine involves using herbal preparation for safe and effective use (WHO guidelines). Historically, plants provided a significant source in encouraging novel drug compounds, making substantial contributions to human health. Traditional medicine, including plant extracts, herbal formulation, and folk remedies, continues to play an essential role in health coverage for over 80% of the world's population, especially in developing countries.[1]

In recent years, about 43% of the total deaths in developing countries have been due to infectious diseases. Fungal infections are estimated to occur in over a billion people each year, and recent evidence suggests the rate is increasing.[2-5]

Medicinal plants play a vital role against the various bacterial pathogens followed from ancient times[6] Several plant extracts have numerous health-related effects such as antibacterial, antimutagenic, anticarcinogenic, antithrombotic, and vasodilatory activities.[7] Recently, there are several plants such as Murrayakoenigii, Syzygiumaromaticum, Piper nigrum, Ocimumtenuiforum, Laurusnobilis, Cinnamomumzeylanicum, Phyllanthusniruri, Cuminumcyminum, Trilobatum sp., and Hibiscus rosa Sinensis which showed antibacterial activity in methanol, aqueous, ethanol, acetone, and petroleum ether extracts against gram-positive and gram-negative bacteria.[8]

Over the past decade, herbal medicine has become a topic of augmented global importance, impacting both world health and international trade. In terms of world health, traditional medicinal plants continue to play a central role in the healthcare systems of large proportions of the world's population.[9] This is particularly true in developing countries, where traditional systems of medicine have a long and interrupted history of use. Recognition and development of traditional medicinal plants' medicinal and economic benefits are on the increase in both developing and industrialized countries. However, it varies significantly from region to region.[10] Since their discovery, antimicrobial drugs have proved remarkably

[1]RIMT University, Mandi Gobindgarh, Punjab, India

[2]Maharaja Agrasen University, Baddi, Himachal Pradesh, India

Corresponding Author: Jyoti Gupta, RIMT University, Mandi Gobindgarh, Punjab, India. E-Mail: jyotipharma175@gmail.com

How to cite this article: Gupta J, Garg R, Mittal S. *In Vitro* Anti Microbial Activity of *Roylea elegans*, *Chrozophora tinctoria*, and *Daphniphyllum himalaense*. Indian J. Pharm. Biol. Res. 2021;9(3):1-9.

Source of support: Nil

Conflict of interest: None.

Received: 24/06/2021 **Revised:** 28/08/2021 **Accepted:** 05/09/2021 **Published:** 30/09/2021

effective for controlling bacterial infections. However, it was soon evident that bacterial pathogens were unlikely to surrender unconditionally because some pathogens rapidly became resistant to many of the first discovered effective drugs.[11] The antimicrobial compounds from plants may inhibit bacterial growth by different mechanisms than those presently used as antimicrobials and may have a significant clinical value in treating resistant microbial strains.[12]

LITERATURE REVIEW

The bacteria are microscopic organisms with relatively simple and primitive forms of prokaryotic type. Danish Physician Christian Grams discovered the differential staining technique known as Gram staining, which differentiates the bacteria into two groups "Gram-positive" and "Gram-negative," Gram-positive bacteria retain the crystal violet and resist decolorization with acetone or alcohol and hence appear deep violet;. In contrast, Gram-negative bacteria, which lose the crystal violet, are counter-stained by safranin and hence appear red.

Since ancient times, the herbal combination has been a traditional remedy to cure several illnesses. Herbs are combined to give the benefits needed from each, some to boost others, some to boost the body with healing energies. These combinations can usually be in any form you choose - teas, tinctures, capsules, etc.

A Hydrogel is a soft, solid, or solid-like material consisting of two or more components, a liquid, present in substantial quantity; gels may appear more solid-like than liquid-like. Hydrogels are also composed of hydrophilic polymer strands, but they are cross-linked, enabling them to swell while retaining their three-dimensional structure. Hydrogels are gels systems in which an insoluble polymer immobilizes water. One reason for the interest in hydrogels as a component of drug delivery systems is their relatively good compatibility with biological tissue. Instead of conventional creams, hydrogels have been formulated for better patient compliance. These hydrogels have moisturizing properties; therefore, scaling and dryness are not expected with this drug delivery system. Also, it has shown better absorption than conventional cream formulations.

Common gelling agents are, e.g., Tragacanth, Sodium Alginate, Pectin, Starch, Gelatin, Cellulose Derivatives, Carbomer, and PolyVinyl Alcohol.

The details of three medicinal plants are listed below

1. *ROYLEAELEGANS*

Plant Profile

Synonym: Royleacinerea (D.Don) Baill.

Common names: Patkarru (Hindi); Kaur, Kauri (Punjabi); Titpati, Karanoi (Kumaoni); Karui (Garhwali).

Family: Lamiaceae

Distribution: Subtropical Western Himalaya, from Kashmir to Nepal, 2,000-5,000 ft

Plant description: It is an erect, lemon-scented hoary shrub, 1.0-1.6 m high. Leaves are shortly stalked, hairy, ovate, 2.5-3.8 cm, acute or acuminate, crenate with base rounded. Flowers are white tinged with pink, in short, axillary umbellate whorls; calayx-tube cylindrical, 10-nerved; lobes 5, erect, oblong, net-veined, obtuse; corolla hairy, hardly longer than the calyx; stamens 4, unequal pairs, ascending under the lip; nutlets are ovoid-oblong, obtuse and smooth.[13,14]

Figure 1: *Royleaelegans*

Chemical constituents: Till now, this plant is not explored for its chemical constituents; only phytochemical screening is reported in the presence of carbohydrates, alkaloids, and terpenes.[15]

Ethnobotanical uses

The infusion of leaves is used as a bitter tonic, and febrifuge[13] Shoots are crushed and eaten with salt to strengthen the liver and extract leaves and shoots used in scabs and skin infections.[16]

The aerial parts of the plant are widely used against liver disorders, and in some tribal areas, leaves are beaded into beads and garlanded by infants to avoid jaundice.[17]

CHROZOPHORATINCTORIA

Plant profile

Synonyms: Croton tinctorium, Crozophoratinctoria.
General names: Dyer's litmus, southern chrozophora, croton, Dyer's crotone, turnasole.
Species name: Chrozophoratinctoria (L.) Juss.
*Maltese name:*Turnasol.
Family: Euphorbiaceae.
Tinctoria: Indicates a plant used in dyeing or has sap that can stain.

Botanical Data

Plant Structure: Characteristic Growth Form Branching Surface Description Erect: Upright, vertically straight up well clear off the ground. Moderately Branched: Considerable number of secondary branches along the main stem.
Stellate: Hairs that radiate out from a common point like the points of a star.
Leaves: Characteristic Arrangement Attachment Venation Description Alternate: Growing at different positions along the stem axis.
Stalked/Petiolate: Hanging out by a slender leaf-stalk.
Pinnate venation: Lateral veins which diverge from the midrib towards the leaf marhins.
Leaf Color: Ash-Green is easily spotted in its habitat.
Flowers: Simple, elongated, indeterminate cluster with stalked flowers. They are tightly close to each looking like a short spike. The male and female flowers are very small (1 mm) and so inconspicuous. The male flowers have five yellow petals and a cluster of five black panthers at the center. The female flowers have no petals, only a globular ovary (enclosed by 10 sepals) with three yellow styles that each split into two.
Inferior: Ovary situated below the flower parts (the calyx, corolla, and androecium). In other words, these are attached above the ovary.
Seeds: Characteristic, Ovoid to Teeth shaped. The shape is more or less oval, but cross-sectional circumference has angular edges (hence not perfectly round). Seed possess a blunt tip.4 mm.

Plant Description

Life Cycle: Annual
Habitat: Dry fields and waste ground. Common in fallow fields too.
Plant height: 30-50 cm.
Flowering Time: July - September
The plant has an ash-gray green appearance because it is densely covered with white, wool-like (tomentose) hairs. The hair is described as stellate (star-shaped) since groups

Figure 2: *Chrozophoratinctoria*

of hair bristles are arranged as radiating out from a common point, and so they have the shape of a pointed star. The plant produces a few simple branches starting at least one-third up the plant height. The basal stem is thin and yellow-amber.

Leaves grow alternately along the stem and are not found in large numbers per plant. The mature leaves have a long petiole (longer than the leaf length) and a rhombic to ovate shape. Leaf margins are sinusoidal (wavy) in a perpendicular plane to the lamina plane. The plant is monoecious, producing male and female flowers separately, which both are tiny (1-2 mm) and therefore inconspicuous. The male flowers have a 5-sepal calyx, five yellow petals, and a cluster of five central stamens with dark or black anthers. The female flowers have a 10-sepal calyx around a spherical ovary, no petals, and three styles, each subdivided into two stigmas. The male and female flowers outgrow as a raceme at the top of the branch, but they are so densely packed that they appear to be a spike. Male flowers are above the basal female flowers in the spike-like raceme. A small-sized species of ants pollinate Flowers.

ChrozophoraTinctoria is a dye plant native to Syria used as an illuminator pigment and a Blue food coloring. It was sold commercially in Europe for staining "clothlets" or Rags. The term turnsole came to mean any such rag used as a coloring agent, whether the original source of color was the herb Turnsole or some other source such as woad, Cochineal, or grape juice. The M. Webster online dictionary defines turnsole as a European herb (*Chrozophoratinctoria*) of the spurge family with juice turned blue by ammonia and a purple dye obtained from it. Turnsole is a purple dyestuff, formerly used as a food coloring, especially for jellies. It is obtained from the fruits of *Chrozophoratinctoria* L., a member of the spurge family native to the Mediterranean. The green juice of the fruits was pressed out with a roller, and coarse linen rags were allowed to soak it up. These were dried, and on exposure to air or ammonia fumes, turned a beautiful purple color. Turnsole was once much cultivated in the South of France, but is rarely met with in modern times due to the widespread use of synthetic dyes.

Toxicity: Few reports such as those stated by the US Food and Drugs Administration indicate that the plant has toxic properties to some farm animals and even to man. Furthermore, a document titled "Sand Dune Vegetation of Cholistan (Pakistan) and Some Control Measures Against Wind Erosion," written by Dr. Mirza Hakim Khan, lists Chrozophoratinctoria as an unpalatable poisonous plant and needs attention for its control. Toxicity evidence on this plant needs to be fortified by more research work.

DAPHNIPHYLLUMHIMALAENSE

Plant Profile

Plant name: Daphniphyllumhimalaense ssp. macropodum.

Description: Daphniphyllum is the sole genus in the flowering plant family Euphorbiaceae. The genus includes about 25–30 species, all evergreen shrubs and trees mainly native to east and southeast Asia and found in India, Sri Lanka, and Australia. All species are dioecious; that is, male and female flowers are borne on different plants.

Botanical descriptions

Diagnostic characters: Twigs glabrous. Leaves clustered at apex of the twigs, simple, alternate, glabrous, below glaucous, young leaves blackish when drying. Flowers unisexual on separate trees, petals absent. Fruits an ovoid drupe, turning purple, shiny, and blackish when ripens. Bifurcate recurved styles and calyx persistent.

Habit: Deciduous medium tree up to 25 m tall. Branches ascending to the main trunk.

Figure 3: *Daphniphyllumhimalaense*

Trunk and bark: Trunk straight. Bark smooth, lenticelled, light grey-brown, inner bark brown, wood cream or white, soft.

Branches and Branchlets or Twigs: Twigs terete, irregular, lenticelled, with leaf scars, purplish green, glabrous.

Exudates: Exudate absent.

Leaves: Leaves simple, alternate, and spiral, 5-20 by 1.5-3 cm, lanceolate or elliptic, apex acute to slightly acuminate, base acute, margin entire, recurved, blade leathery, dark green above, glaucous below. Young leaves drying blackish.

Midrib flat above, primary vein single, pinnate, secondary veins oblique to the midrib, widely parallel, tertiary veins finely reticulate.Petiole long, slightly caniculated, glabrous.

Inflorescences or flowers: Flowers unisexual on different trees, small, grouped in racemose inflorescence, axillary, pedicel longuer than 0.5 mm., petals absent. Female flowers styles 2, recurved, calyx persistent.

Fruits: Drupe ovoid or ellipsoidal, 1–1.5 by 0.8 cm, shiny, bluish-green, becoming purple when ripen, calyx and bifurcate styles very small persistent.

Seeds: Seed one.

Daphniphyllum is dioecious; the male flowers are on one plant and female flowers on another. Single plants fail to fruit. For a good set, planting five or more individuals in close proximity is a good idea. We have run several seed sources and find the seed germinates readily after the fruit, a single-seeded drupe, has been cleaned. Cutting propagation has been another matter – we aren't at zero success, but we're close to it. The variability in leaf shape, petiole-midrib color, and plant structure is enough to justify further propagation trials. Plant growth in the first few years is slow, but the species is quite durable once established. Daphniphyllum requires a part-shade to a shady location. While the plant grows best in neutral to mildly acid soils, it is tolerant of higher pH soils.

MATERIAL AND METHODS

Chemicals and Reagents

Standard drug Cefixime as gift sample from Cipla Laboratories. All other chemicals and reagents are of LR and AR grade, respectively.

Plant Materials

- Fresh aerial parts of the plant were collected from the foothills of the Himalayas region (Distt– Pithoragarh (Uttarakhand)) and were authenticated by Ms. Jyotigupta, Assistant Professor, IEC school of pharmacy, Solan. The collected aerial parts of the plant was made thoroughly free from any foreign organic matter, dried under shade and powdered.
- Fresh aerial parts of *Chrozophoratinctoria* L were collected from the Pauri region of Garhwal Himalayas, Uttarakhand, and authenticated by Ms. Jyotigupta, Assistant Professor, IEC school of pharmacy, Solan. The collected aerial parts of the plant were made thoroughly free from any foreign organic matter, dried under shade, and powdered.
- Fresh aerial parts of *Daphniphyllumhimalaense* were collected from the Mussoorie region of Dehradun, Uttarakhand, and were authenticated by Ms. Jyotigupta, Assistant Professor, IEC school of pharmacy, Solan. The collected aerial parts of the plant were made thoroughly free from any foreign organic matter, dried under shade, and powdered.

Preparation of Extract

- The Hydroalcoholic extract was prepared using 50% aqueous ethanol by triple maceration process for 48 h each time. The extract was filtered and concentrated. The concentrated extract was further used for phytochemical screening, establishing a TLC profile, and screening for activity.
- Coarsely powdered aerial parts of *Chrozophoratinctoria* L were subjected to maceration process using hydro-alcohol (50 %) for 46 hrs each in triplicate. The solvent was distilled out, and the concentrated extract was further used for phytochemical screening, establishing a TLC profile, and screening for activity.
- Aerial parts of *Daphniphyllumhimalaense* were subjected to a triple maceration process using hydro-alcohol (50 %) for 46 hrs each. The solvent was distilled out, and the concentrated extract was further used for phytochemical screening, establishing a TLC profile, and screening for activity.

Preliminary Phytochemical Screening of all Extracts

The hydroalcoholic of three medicinal plants were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids[12]

Test for Alkaloids: To hydroalcoholic extract of *R. elegans*, 2 N HCl was added, and preliminary screening of alkaloids was tested. After this, confirmatory test were done by using the standard acidification and basification procedures. Following tests were performed to detect the presence of alkaloids.

Dragendorff test: 1ml of the extract, 1ml of Dragendorff's reagent was added; an orange, red precipitate indicated the presence of alkaloids.

Mayer's test: To 1ml of the extract, 2 mL of Mayer's reagent was added, a cream-colored precipitate revealed the presence of alkaloids.

Wagner's test: To 1ml of the extract, 2mL of Wagner's reagent was added; the formation of a reddish-brown precipitate indicated the presence of alkaloids.

Hager's test: To 1ml of the extract, 3 mL of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

Test for Carbohydrates: Hydroalcoholic extract was dissolved in methanol (50%), and the following tests were determined to detect carbohydrates.

Molisch test: To 2 mL of the extract, 1ml of the alpha-naphthol solution was added, and then, concentrated sulphuric acid was passed through the sides of the test tube. Purple or reddish-violet color at the junction of the two liquids revealed the presence of carbohydrates.

Fehling's test: To 1 mL of the extract, an equal quantity of Fehling's solution A and B was added, and the formation of red brick precipitate indicated the presence of carbohydrates.

Benedict's test: To 5 mL of Benedict's reagent, 1ml of extract solution was added and boiled for 2minutes and then cooled. The formation of a red precipitate showed the presence of carbohydrates.

Barfoed's test: To 5 mL of Barfoed's solution, 1ml of extract solution was added and boiled for a few minutes; formation of a red precipitate of copper oxide was formed and confirmed the presence of carbohydrates in the test extract.

Test for Glycosides: The hydroalcoholic extract was dissolved in methanol (50%), and then sulphuric acid was added dropwise for hydrolysis. A few ml of chloroform was added and shook well. The aqueous part was tested for sugar moiety, indicating the presence of sugars for preliminary screening of glycosides. Following tests are performed for the detection of glycosides.

Legal test: The extract was dissolved in pyridine, and a freshly prepared sodium nitroprusside solution was added to make it alkaline. The formation of pink to red color showed the presence of glycoside.

Baljet test: To 1 mL of the test extract, 1ml sodium picrate solution was added, and a change in yellow to orange color revealed the presence of glycoside.

Borntrager's test: A few ml of diluted sulphuric acid was added to 1 mL of the extract solution. The solution was boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was separated and treated with 1 ml ammonia. The formation of the red color showed the presence of anthraquinone glycoside.

Modified Borntrager's test: 5% $FeCl_3$ was added to 1-mL of extract solution with a few ml of diluted sulphuric acid. The solution was boiled, filtered and the filtrate was extracted with chloroform. The chloroform layer was separated and treated with 1ml ammonia. The formation of the red color showed the presence of anthraquinone glycoside.

Keller Killiani test: The extract was dissolved in acetic acid containing traces of ferric chloride and transferred to a test tube containing sulphuric acid. At the junction, the formation of a reddish-brown color, which gradually becomes blue, confirmed the presence of glycoside.

Test for steroids and sterols: The hydroalcoholic extract was dissolved in methanol (50%) The following tests were done to detect steroids and sterols.

LibermannBurchard test: The extract was dissolved in 2ml of chloroform in a dry test tube and few drops of acetic anhydride were added, followed by concentrate sulphuric acid. The solution becomes red, then blue, and finally bluish-green, indicating steroids' presence.

Salkowski test: The extract was dissolved in chloroform, and an equal volume of concentrate sulphuric acid was added. Formation of bluish-red to cherry red color in chloroform layer and whereas the acid layer assumes marked green fluorescence, represented the steroid and sterol components, in the tested extract.

Test for saponins: The extract was diluted with 20ml of distilled water and shook in a graduated cylinder for 15 minutes. The formation of foam indicated the presence of saponins.

Test for flavonoids: Hydroalcoholic extract was dissolved in methanol (50%), and the following tests were performed to detect flavonoids.

Shinoda test: To 1ml of the extract, magnesium turnings were added, and 1-2 drops of concentrated hydrochloric acid were dropped from sidewise. The formation of the pink or red color showed the presence of flavonoids.

To 1 mL of extract, 1-mL of ferric chloride was added; the formation of brown color confirmed the presence of flavonoids.

*Test for tannins and phenolic compounds***:** hydroalcoholic extract was dissolved in methanol (50%) to detect tannins, and the following tests were performed.

To 1-mL of extract, 5% neutral ferric chloride was added, and a dark blue color product showed the presence of tannins.

To 1-mL of extract, a few mL of gelatin solution was added, and a white precipitate revealed the presence of tannins and phenolic compounds.

To 1 mL of the extract, lead tetra acetate was added, and a precipitate production showed the presence of tannins and phenolic compounds.

Test for tri-terpenoids: To detect tri-terpenoids, two or three granules of tin metal were dissolved in 2 mL of thionyl chloride solution, and then, 1-mL of the extract was added into the test tube. The formation of a pink color indicated the presence of tri-terpenoids.

Test for proteins and amino acids: For the detection of proteins and amino acids, the hydroalcoholic extract was dissolved in methanol (50%), and the following tests were performed.

Biuret test: To 1-mL of the extract, 1-mL of 40% sodium hydroxide solution and 2 drops of 1% copper sulfate solution was added. The development of violet color indicated the presence of proteins.

Ninhydrin's test: Two drops of freshly prepared 0.2% Ninhydrine reagent were added to the extract solution and then heated. The development of a blue color revealed the presence of proteins and amino acids.

Xanthoproteic test: To 1ml of the extract, 1-mL of concentric nitric acid was added. The formation of white precipitate confirmed the presence of amino acid.

Test microorganisms

Tested bacterial strain *Escherichia coli* were gift samples from the Department of Microbiology, Kalandi Pharmaceuticals, Selaqui, Dehradun, India**.**

Formulation of Hydrogel

Herbal hydrogel formulation was prepared, using sodium alginate as gel base. 2% w/v of sodium alginate was dispersed in 70 mL of distilled water and was allowed to swell. 10 gm of extract mixture from all the above-mentioned plants was dissolved in 20 mL of ethanol which also acts as a penetration enhancer and was added in the previous mixture, followed by stirring, using mechanical stirrer for 10 minutes.[18] Further, 1.5% of $CaCl_2$ was added to enhance cross-linking of polymers and was stirred for half an hour to allow polymer.[19] 10% glycerin was added as a humectant and as a plasticizer. Finally, the gel was allowed to stand for 48 hrs to settle and stored in appropriate storage conditions.

Media preparation

The agar media was prepared using peptone (1 g), agar (5 g), yeast (0.3 g), sodium chloride (0.5 g), and dissolving these ingredients in water q.s. to make 50 mL. The media were then sterilized by autoclaving.

Evaluation of *in-vitro* antibacterial activity

In-vitro antibacterial activity was evaluated using the disk diffusion method. To determine the antibacterial activity, a 0.2 µL bacterial cultures were poured with a nutrient agar medium (30 mL) in different Petri plates (90 mm). Sterilized filter paper discs (Whatman No. 1; 6 mm in diameter) soaked in different beakers containing the standard drug, cefixime (200 mg), and different hydroalcoholic extracts (5 and 10 µL) were taken out with sterilized forceps and air-dried and placed on plates with the different organisms.

The plates were incubated at 37°C for 24 h for bacterial strains, for 2 days at 37°C. After incubation, the inoculated plates were observed for zones of inhibition in millimeter diameter using a transparent ruler.

RESULTS

Preparation of Hydrogel

Herbal hydrogel formulation was prepared using 2% w/v of sodium alginate used as a gelling agent and was allowed to stand for 48 hrs to settle and stored in appropriate storage conditions. The amount of hydrogel was collected about 100 mL.

Preliminary Phytochemical Screening of Extracts

Following metabolites were observed during phytochemical screening (Table 1).

Evaluation of *in-vitro* antibacterial activity

The in-vitro antibacterial activity was evaluated using disk diffusion method. The hydrogel (5 and 10 µgm/mL) was compared to the standard drug cefixime (200 mg).

The results are mentioned in Table 2, and Figures 4 and 5.

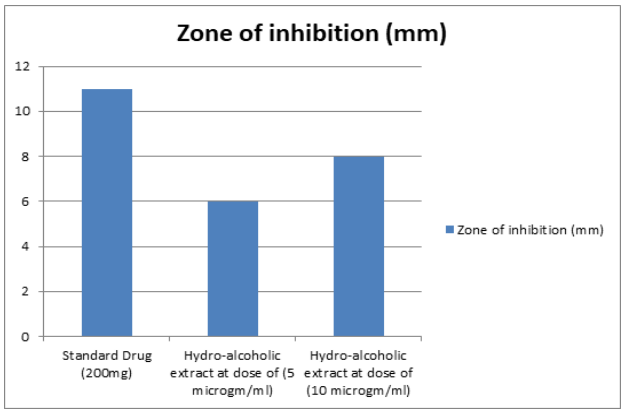

Figure 4: Results showing the in-vitro antibacterial activity of hydroalcoholic extract of *Chrozophoratinctoria*

Table 1: Preliminary Phytochemical Screening of hydroalcoholic extract of *Chrozophoratinctoria.*

SN.	*Chemical constituents*	*Royleaelegans*	*Chrozophoratinctoria*	*Daphniphyllumhimalaense*
1.	Alkaloids	Present	Absent	Present
2.	Glycosides	Absent	Absent	Present
3.	steroids and sterols	Present	Absent	Absent
4.	Saponins	Present	Present	Present
5.	Flavonoids	Absent	Present	Present
6.	Tannins and phenolic compounds	Absent	Present	Present
7.	Tri-terpenoids	Absent	Present	Present
8.	Proteins and amino acid	Absent	Present	Present

Table 2: Results showing the *in-vitro* antibacterial activity of hydroalcoholic extract of *Chrozophoratinctoria*

Zone of inhibition (mm)			
Bacteria	*Standard Drug (Cefixime 200 mg)*	*Hydroalcoholic extract at a dose of (5 µm/mL)*	*Hydroalcoholic extract at a dose of (10 µgm/mL)*
Escherichia coli	11	06	08

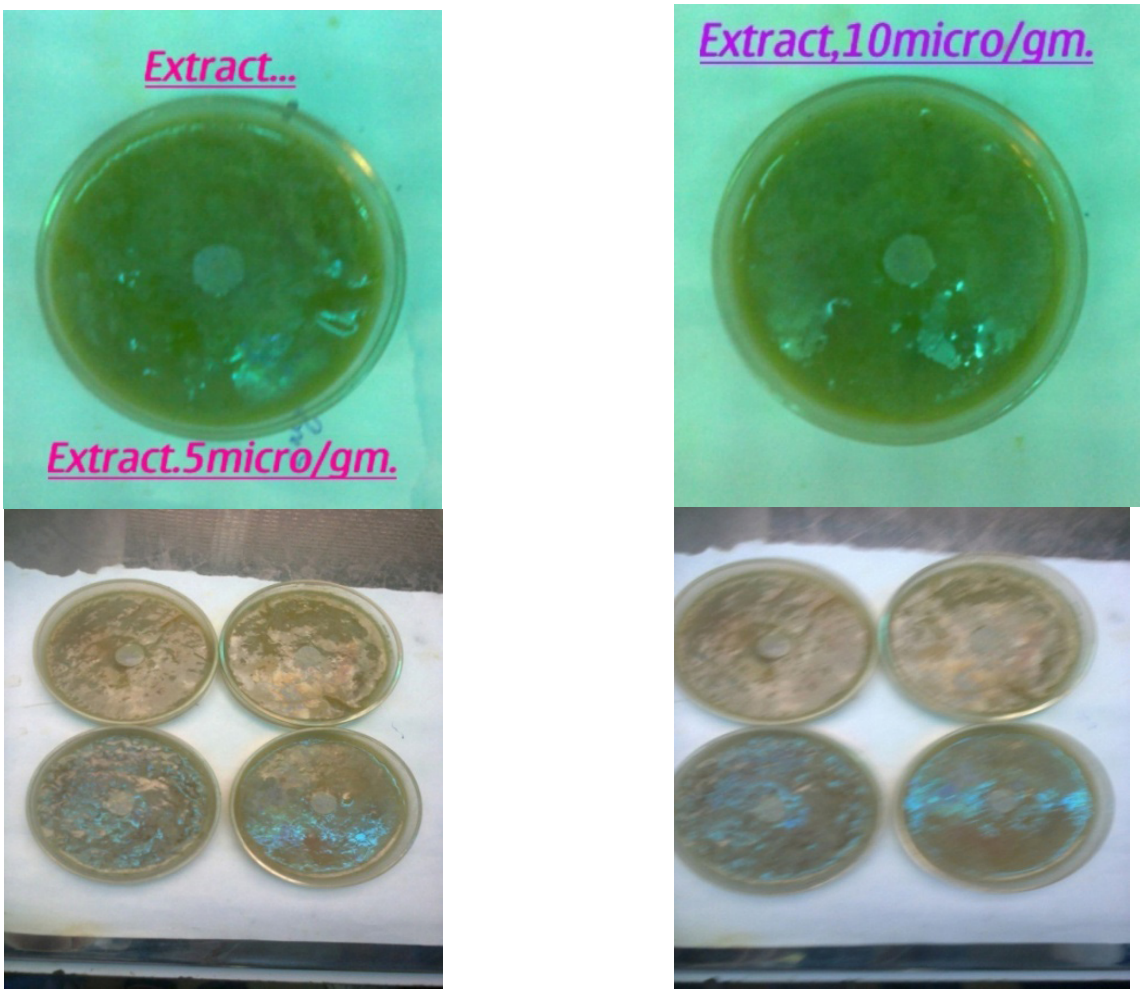

Figure 5: Results showing the *in-vitro* antibacterial activity of hydroalcoholic extract of *Chrozophoratinctoria*

DISCUSSION AND CONCLUSION

The hydrogel containing different plant materials shows the significantly *in-vitro* antibacterial activity as compared to standard drug cefixime. The different concentrations (5, 10 microgm/mL) of hydrogel showed a remarkable decrease in the growth of bacteria *E. coli* as compared to the standard. The highest dose of hydrogel (10 microgm/mL) showed maximum results compared with the standard. The data indicated that the extracts of *Hydrogel formulation* showed significant antibacterial activity, which supports the traditional use of all three listed medicinal plants.

In conclusion, the medicinal plants were selected for in-vitro antibacterial activity depending on their traditional use. The hydroalcoholic extract was prepared using the maceration process, and the preliminary phytochemical screening was performed individually on each plant.

The hydrogel was prepared using sodium alginate as a gelling agent of hydroalcoholic extract of all mentioned extracts. In-vitro antibacterial activity was performed on hydrogel on different dose levels and was compared with standard drugs.

REFERENCES

1. Igbinosa OO, Igbinosa O, Aiyegoro OA. Antimicrobial activity and phytochemical screening of stem bark extracts from Jatrophacurcas (Linn). Afr. J. Pharm. Pharmacol. 2009; 3(2):58-62.
2. Zaika LL. Spices and herbs: Their antimicrobial activity and its determination. J. Food. Safety. 1988;9:97-118
3. Bidlack WR, Omaye ST, Meskin MS, Topham DKW. 2000;. Phytochemicals as Bioactive Agents. CRC press, Boca Raton, FL.
4. Soniya M, Kuberan T, Anitha S, Sankareswari P, 2012, *In vitro* antibacterial activity of plant extracts against Gram positive and Gram negative pathogenic bacteria, International Journal of Microbiology Immunology Research 2(1), 001-005.
5. Zhang X. Regulatory Situation of Herbal Medicines. A Worldwide Review. WHO: Geneva, Switzerland, 1998. 1–5.
6. Cowan MM. Plant products as antimicrobial agents. ClinMicrobiol Rev 1999; 12(4): 564–582.
7. Harborne JB. Phytochemical Methods, 3rd ed. London: Chapman Hall, 1998. 1–302.
8. Sattar FM, Harraz FM, Sabah H, Gayed E. Antimicrobial Activity of Extracts of some Plants collected from the Kingdom of Saudi Arabia, JKAU: Med. Sci. 2008;15: 25-33.
9. Vats M, Sharma N, Sardana S. Antimicrobial activity of stem bark extracts of *Nyctanthesarbortristis* linn. Int J PharmacogPhyto Res. 2009;1:12–4.
10. Prakash V, Aggarwal A. Traditional uses ofethanomedicinal plants of lower foot hills of Himanchal Pradesh-I. Indian Journal of Traditional Knowledge. 2010;9,519-521.
11. Tiwari JK, Ballabha R, Tiwari P. Diversity and Present Status of Medicinal Plants in and around Srinagar Hydroelectric Power Project in Garhwal Himalaya, India: Needs for Conservation. Researcher. 2010;2:50-60.
12. Upadhyay G, Kamboj P, Malik J. Pharmacognostical studies and Evaluation of Quality Parameters of *Royleaelegans* WALL.(aerial parts International Journal of Research in Pharmaceutical and Biomedical Sciences. 2011;2(4):1678-1685.
13. Patil S K, Salunkhe V, Ghumte D, Mohite S, Magdum C. comparative studies on anti-inflammatory activity of hydrogels containing herbal extracts. 2012;2(4):612-616.
14. Akerele O. Medicinal plants and primary health care: an agenda for action. Fitoterapia 1988;59:355–36315
15. WHO General guidelines for Methodologies on Research and Evaluation of herbal medicines. 2000;14:237.
16. Chaudhari AC, Dixit SK. Quality Control and Standardization of Herbal Raw materials and Traditional remedies, Eastern Pharmacist. 1995;3:23.
17. Patani A. Indian Herbal Pharmacopoeia Revised new eddition Indian drug manufacturer association publications, 2002; 550.
18. World Health Organization, Research guideline for evaluating the safety and efficacy of herbal medicine. Available from: www.WHO.org