World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

ANTIBACTERIAL ACTIVITY OF POLYHERBAL FORMULATION AGAINST URINARY TRACT INFECTION

Preyenga R.*, Kayalvizhi B.[1] and Alifiya Sultana H.[2]

[*,1]Evolute Bioscience, Trichy-620003.
[2]Bishop Heber College (Autonomous), Affiliated to Bharathidasan University, Trichy, Tamil Nadu - 620017.

Corresponding Author: Preyenga R.
Evolute Bioscience, Trichy-620003.

Article Received on 30/06/2023 **Article Revised on 20/07/2023** **Article Accepted on 10/08/2023**

ABSTRACT

Medicinal plants have been extensively used for various ethno pharmacological properties from ancient days. Poly herbal formulation of the medicinal plants having antimicrobial property were assessed using five different plant species includes holy basil, curry leaves, bishop's weed creeper, betel and neem leaves. This poly herbal formulation was tested for the treatment of urinary tract infections. The test organism was characterized by different biochemical tests and phytochemical screening was performed for the aqueous and ethanolic polyherbal extract. The results were found to be promising for the poly herbal aqueous extract against the test organism isolated from urinary tract infected patients and the extract inhibited the test organism to a greater extent which confirms that it could be potentially employed as the antimicrobial agent for UTI infections.

KEYWORDS: Urinary tract infection, polyherbal extract, medicinal plants, phytochemicals.

INTRODUCTION

Plants are predominantly photosynthetic eukaryotes and possesses versatile pharmacological uses. Each and every plant species have got its significant properties which could be utilized for many applications.[1,2] The plant contains phytochemicals which includes Alkaloids, Flavanoids, Terpenoids, Tannins, Glycosides, Saponins were found to be the promising phytocompounds responsible for the pharmacological properties of medicinal plants.[3,4] Urinary tract infections (UTI) has become one of the most common diseases affecting women worldwide.[5,6,7] Nearly 1 in 10 of the female individuals has been suffering with this infection. It was seen that the urinary tract infections were caused due to many bacterial infections.[8] Out of all the microorganisms, the *Escherichia coli* was seen as the major causative elements for UTI infections in women. Antibiotics were usually administered for the treatment of UTI infection that combats or inhibits the bacterial growth in the lining of urinary tract.[9] Sometimes the bacteria become resistant to the antibiotics administered and they survive in the urinary tract causing itching and irritation during urination. Instead of employing antibiotics, the infections could be treated naturally with the administration of extracts of medicinal plants containing phytochemicals.[10,11] There are many different plants exhibiting antibacterial activity such as Neem leaves, Curry leaves, Betel leaves, Holy Basil and Bishop's weed creeper etc., All these plants were found to exhibit significant antibacterial activity against both gram positive and gram negative organism.[12] All these plants showing antibacterial activity were taken together and Poly herbal formulation was made. The prepared poly herbal formulation was used for the treatment of urinary tract infection.

MATERIALS AND METHODS

Sampling, Isolation and Screening

The bacteria used in this study were collected from urinary tract infected patients. UTI sample were treated with Poly herbal formulated plant extract. The medicinal plants such as *Azadirachta indica*, *Coleus aromaticus*, *Murraya koenigii*, *Ocimum tenuiflorum* and *Piper betle* were used to prepare the polyherbal formulation. All these five different plants were taken in equal amounts, washed with distilled water and shade dried for 3-4 days in room temperature for 60℃. After drying these leaves were crushed and grinded into powder. Then the powdered sample were weighed in equal amounts and dissolved in distilled water and ethanol to collect the aqueous and ethanol fraction respectively. To prepare the aqueous extract, the plant leaves were taken in the ratio of 1:1:1:1:1 and finely grinded which is dissolved in distilled water and heated to 60℃ for 20 minutes. To prepare the ethanolic extract of the plant, the dried plant leaves were taken and dissolved in 70% ethanol for 100mL and kept at normal room temperature for 24h without heating. The prepared aqueous and ethanolic

extract were used for the study of antibacterial activity in agar well diffusion method using nutrient agar medium.

The urine was collected from urinary tract infected patients and the causative bacteria was isolated from it and this test organism was studied for the antibacterial activity. Initially, the sample was streaked (quadrant method) over Mackoney agar medium and in tryptic soy agar medium. The species were mass cultured in tryptic soy broth and this test organism were identified using various biochemical tests.

Initially the gram staining was performed in one clean glass slide and the pure culture of the test organism was smeared into the glass slide. The test organism from UTI sample were analysed using IMViC test i.e., indole test, Methyl red test, Vogus-Proskeur test, simmon citrate test and the results were observed. After that oxidase test, catalase test, starch hydrolysis test, triple sugar iron test were also analyzed for identification of the test organism.

Identification of Test organism

Gram Staining

A thin smear of the bacterial organism was placed over the slide. Using grams iodine, crystal violet, safranin, and decolorizing solution grams staining was performed.

Biochemical Test

Indole Test

0.1g of tryptone was added to 10mL of distilled water and then it was sterilized.

MR VP Test

Glucose Phosphate Broth

About 0.1g of K_2HPO_4, 0.1g of peptone, 0.118g of glucose were added to 20mL of distilled water and sterilized.

Simmon's Citrate Test

Nearly 0.242g of simmon citrate agar was added to 10 mL of distilled water and sterilized. After sterilization, the medium was made into agar slants.

Oxidase Test

The filter paper was dipped with alpha naphthol and the test organism were streaked on kept for 5-10 min. The purple color formation indicates the positive test for oxidase reaction.

Catalase Test

The test organism was smeared in a clean dry test tube and few drops of 3% hydrogen peroxide were added to the slide. The immediate bubble formation indicates the presence of catalase reaction.

Starch Hydrolysis Test

The starch agar medium was prepared for starch hydrolysis and the test organism was smeared in the plate. The plate was then incubated for 24hrs. The zone formation indicates the positive test for starch hydrolysis reaction.

Triple Sugar Iron Test

About 0.3g of triple sugar iron agar were added to 10mL of distilled water and sterilized. Then the plate was incubated for 24 hours after the streaking of test organism over and inside the butt of the agar slant.

Screening of Phytochemicals

The aqueous and ethanolic plant extract were studied for the phytochemical screening. The phenol test, saponification test, carbohydrate test, protein test, glycoside test, flavonoid test, alkaloid test, tannin test, terpenoids test were analyzed for phytochemical screening.

Test for Phenols

The small amount of ethanolic poly herbal extract was taken and 1ml of water was added in a test tube. To the poly herbal extract, 1 to 2 drops of Ferric chloride was added. The formation of blue, green or purple color is indicates presence of phenols.

Test for Carbohydrates

Molisch's Test

A few drops of alpha-naphthol solution were added in 3ml of poly herbal extract in a test tube. It was shaken by the addition of few drops of concentrated sulphuric acid. The formation of violet ring confirms the presence of carbohydrates.

Benedict's Test

Take 3ml of poly herbal extract and few drops of 4% sodium chloride and 1% of copper sulphate solution were added in a test tube. Blue, violet or pink color ring confirms the presence of protein.

Test for Protein

Biuret's Test

1 mL of poly herbal extract was taken to which drops of 4% of sodium hydroxide were added and followed by the addition of 1% copper sulphate. The formation of blue/violet ring indicates the presence of protein.

Test for Glycosides

The small amount of ethanolic poly herbal extract was taken and 1ml of water was added in a test tube. Few drops of aqueous sodium hydroxide was added. The yellow colour change indicates the presence of glycosides.

Test for Flavonoids

1 to 5 drops of concentrated hydrochloric acid were added to the small amount of ethanolic poly herbal extract of the plant material. The appearance of red color indicates the presence of flavonoids.

Test for Alkaloids
2ml of the poly herbal extract was taken in a test tube and 0.2 mL of dilute HCL was added followed by adding 1ml of meyer's reagent in the test tube .Yellowish color indicates the presence of alkaloids.

Test for Tannins
5 mL of poly herbal extract was taken in a test tube and 2mL of 5% of ferric chloride solution were added in it. Greenish black color formation indicates the presence of tannins.

Test for Terpenoids
Salkowski Test
2ml of chloroform was taken in a test tube and 0.5ml of the ethanolic or aqueous poly herbal extract were added. 3ml of concentrated sulphuric acid was added in the test tube. Reddish brown color indicates the presence of terpenoids.

Antibacterial Activity
Agar Well Diffusion Method
The ethanolic and aqueous extract of polyherbal formulation was studied for the antibacterial activity against four different microorganisms such as *Escherichia coli, Klebsiella sp, Streptococcus sp* and *Acetobacter sp.* The Nutrient medium was prepared and sterilized in autoclave for 15-20 minutes and solidified at room temperature. Later, the test organisms were spread over the medium and wells were created and the poly herbal extract was added in different concentrations having volumes such 50µl and 100µl in the respective wells and Ampicillin, a broad spectrum antibiotic was taken as the positive control. The plates were incubated at 37℃ for 18-24 h to observe the zone of inhibition around the created wells. The zone of inhibition (ZOI) was measured in terms of mm.

RESULTS

The poly herbal formulation was prepared from five different antibacterial plants in equal proportion as 1:1:1:1:1. ***Fig 1*** and ***Fig 2*** depicts the polyherbal formulation of medicinal plants and extract preparation respectively. Prepared polyherbal formulation was analyzed for antibacterial activity using Agar well diffusion method. The organisms like *Escherichia coli, Klebsiella sp, Acetobacter sp* and *Staphylococcus sp* were spread over the nutrient agar medium and different concentrations of aqueous and ethanolic extract was added i.e., 50µl and 100µl in the respective wells and showed zone of inhibition (ZOI) as 13 mm and 2 mm and ethanolic extract of 100µl and 50µl as 15 mm and 17 mm respectively for *Escherichia coli* whereas control showed 12 mm. The aqueous extract of 100µl and 50µl showed 20mm and 17 mm and Ethanol extract of 100µl and 50µl and control is found to be resistant against *Klebsiella sp*. The aqueous extract of 100µl and 50µl showed 16 mm and 12 mm and Ethanol extract of 100µl and 50µl showed 12mm and 10mm respectively and control as 20 mm for *Acetobacter sp*. The aqueous extract of 100µl and 50µl showed resistance towards *Staphylococcus sp* and Ethanol extract of 100µl and 50µl as 13mm and 15mm and control is found to be 15mm. The phytochemical screening showed the presence of phenol, sugar, protein, saponin, tannin, terpenoids and flavonoids for aqueous extract. ***Fig 4*** represents the antibacterial activity of extract against test organisms whereas ***Fig 5*** shows the antibacterial activity of extract against UTI sample. The ethanolic extract contains sugar, tannins and terpenoids and the rest phytochemicals were found to be absent or not in the detectable range**.** ***Fig 3*** shows the phytochemical screening of ethanolic and aqueous extract.

The sample from urinary tract infected patients were streaked over Macconkey agar and Tryptic Soy Agar. The colonies thus isolated from Macconkey agar medium were inoculated in Tryptic Soy broth for mass culture. Later, the test organism was identified using various biochemical tests. The gram's staining results showed that it is a gram negative organism having rod shape. The IMViC test reults showed the positive results to Indole, Methyl Red and Citrate utilization test which is found to be negative for Voges-proskeur test. The catalase test was found to be negative. The starch hydrolysis test showed negative results. The sugar fermentation studies of the microorganism showed K/A ++ which is acid slant and acid butt along with the formation of gas. The oxidase test also exhibited negative results for the test organism but the catalase test showed positive results. The identified organism was found to belong to the *Enterobacteriaceae* family based on the biochemical tests. Later, the organism was spreaded over Macconkey agar medium and Tryptic Soy Agar medium to find the antibacterial susceptibility. The aqueous extract of 50µl and 100µl showed 20 mm and 24mm respectively whereas the ethanolic extract of 50µl and 100µl exhibited 15mm and 22mm respectively for the test organism. The control Ampicillin was found to have ZOI as 7 mm against the test organism. The test organism was streaked over Eosin Methylene Blue Agar which showed metallic green colonies after incubation. The test organism was confirmed as *Escherichia coli.* ***Fig 6*** and ***Table 1*** represents the biochemical characterization of test organism isolated from UTI sample.

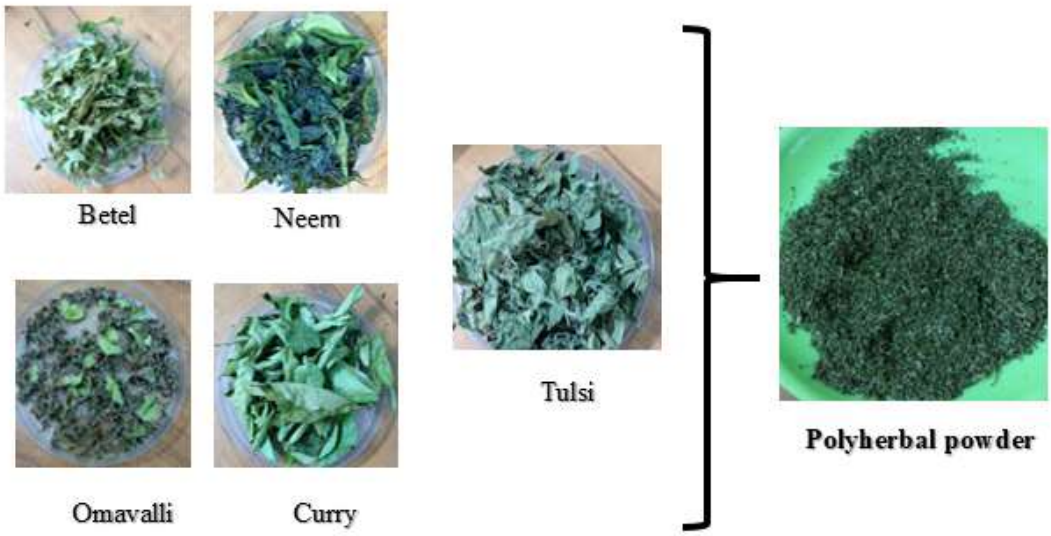

Fig. 1: Polyherbal formulation of medicinal plants.

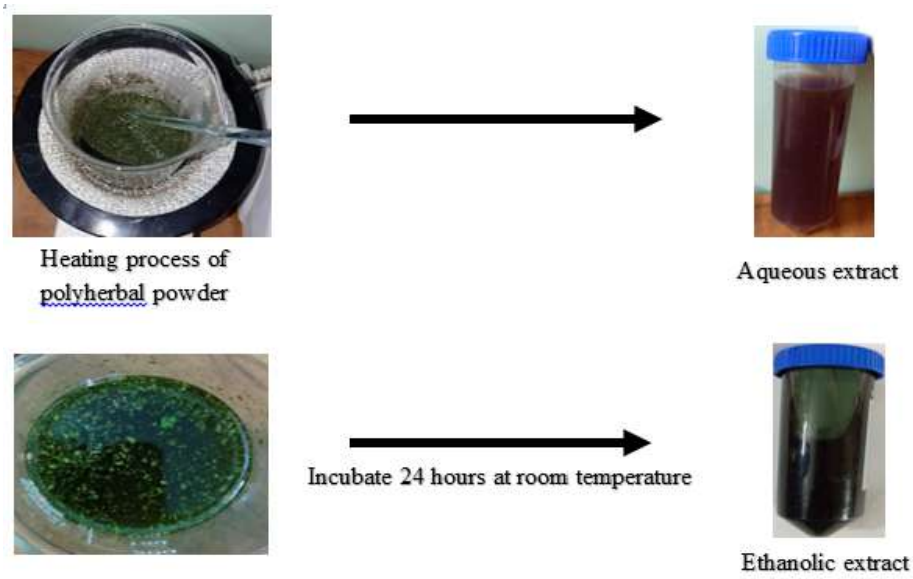

Fig. 2: Aqueous and ethanolic extract preparation.

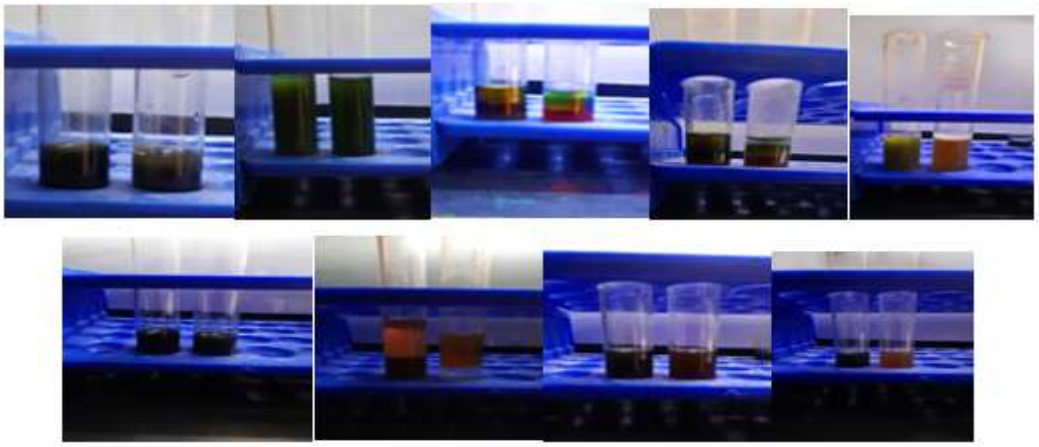

Fig. 3: Phytochemical screening of aqueous and ethanolic extract of polyherbal formulation.

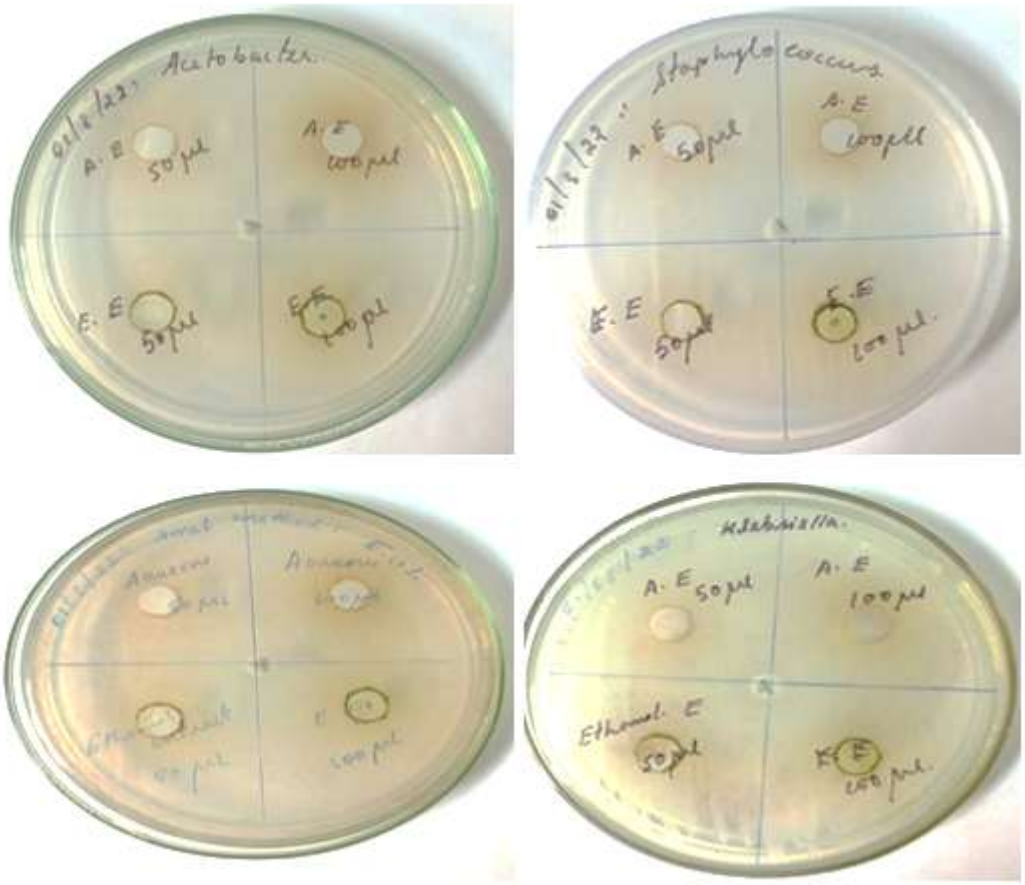

Fig. 4: Antibacterial activity in Nutrient agar for ethanolic and aqueous extract.

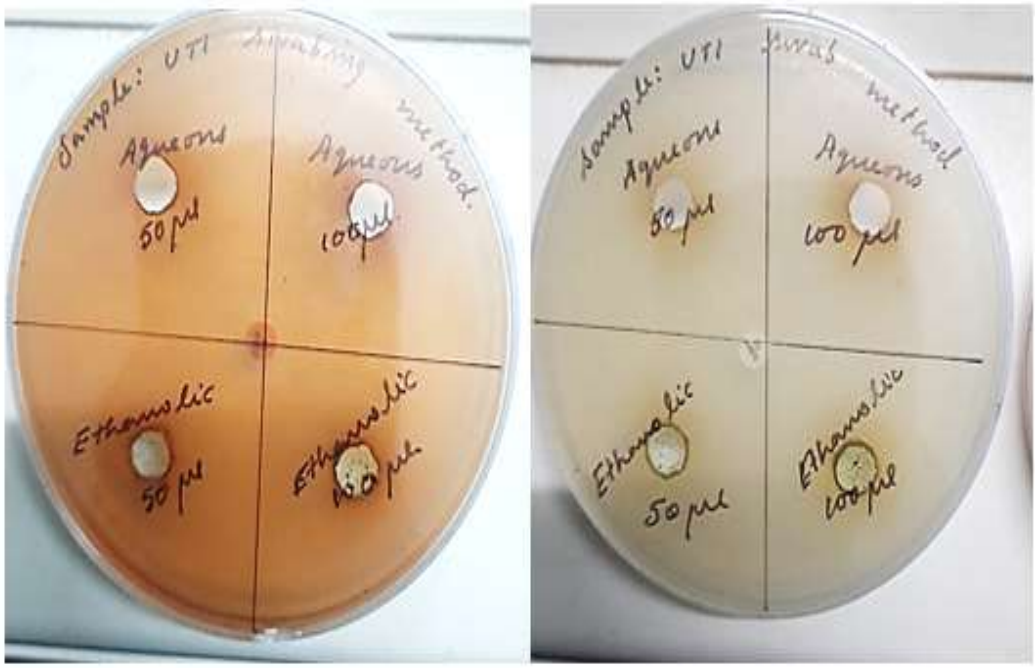

Fig. 5: Antibacterial activity of ethanolic and aqueous extract against UTI sample.

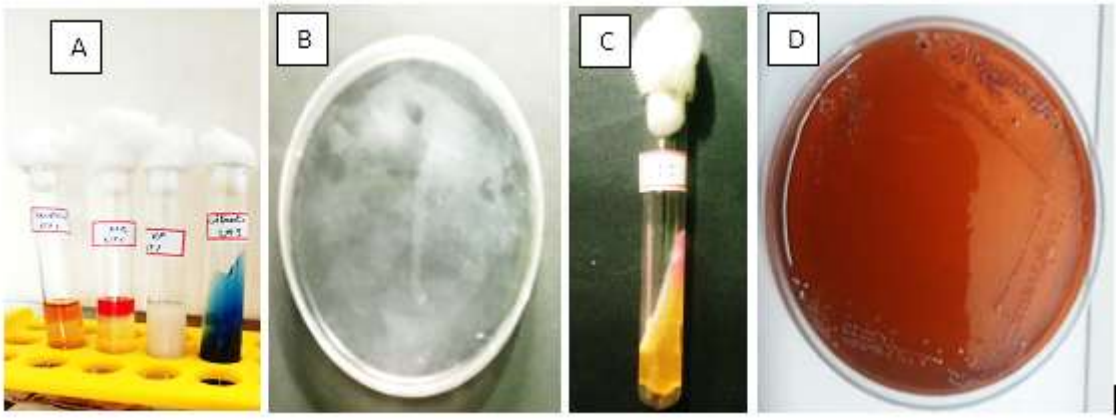

Fig. 6: Characterization of microorganism isolated from UTI sample using A. IMViC test, B. Starch hydrolysis test, C. Triple Sugar iron test, D. Growth in EMB agar plate.

Table:

Table 1: Biochemical characterization of test microorganism isolated from UTI sample

BIOCHEMICAL TESTS	INFERENCE
Indole test	Positive
Methyl Red test	Positive
Vogus-Proskeur	Negative
Simmons citrate test	Negative
Oxidase test	Negative
Catalase test	Positive
Starch hydrolysis test	Negative
Triple sugar iron test	K/A++

DISCUSSION

Polyherbal formulations are mixtures of many plant parts obtained from various plant species and families.[13] These plants/their combinations usually contain an array of bioactive compounds making them suitable for the treatment and management of a variety of disease conditions.[14,15,16] It is generally believed that polyherbal formulations are just effective as the conventional drugs or more potent against diseases when taken alongside conventional drugs.[17] By using herbal combinations, nature provides a balance of ingredients that may act as buffers, synergists or counterbalances, which work in harmony to rid the body of diseases and infirmities.[18] Some herbal extracts have been scientifically proven for efficacy in the treatment of diseases while many others are yet to be investigated.[19]

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, cardiac glycosides, terpenoids and carbohydrates.[20] The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites. They possess biological properties such as apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities.[21,22] Several studies have described the antimicrobial properties of medicinal plants which are rich in phenolic compounds. Natural antimicrobial property mainly come from plants in the form of phenolic compounds such as flavonoid, phenolic acids, and tocopherols.[23]

Our current investigation showed a promising effect on presence of phenolic compounds in almost all the extracts that exhibits antimicrobial activity. Flavonoids are hydroxylated phenolic substances known to be synthesized by the plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms.[24,25] Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall. The present study showed presence of flavonoids particularly when eluted with polar solvents.[26,27]

Antibacterial activity of polyherbal formulation was performed against *E.coli, Klebsiella, Acetobacter and Staphylococcus* and infected urine test sample. In this activity, we are comparing aqueous and ethanolic extract among these aqueous extract was found to be more effective than ethanolic extract.[28]

UTIs have a high economic and social burden and affect large segments of the population, such as children,

pregnant women, healthy pre- and post-menopausal women and patients undergoing catheterization and diabetics. In addition, there is a very high risk of relapse in UTIs, and it is common for these episodes to be caused by the same bacteria or something different. Unfortunately, many of the current treatments fail to resolve UTIs and therefore lead to complications and relapses, and in many cases, patients also experience the adverse effects of medications.[29]

The diagnosis and treatment of recurrent UTI is very complicated in view of the increasing prevalence of antibiotic resistance strains of *E.coli.* Hence, an alternative therapy directed at diminishing bacterial susceptibility and enhancing host defense against UTI rather than using antibacterials is of importance using alternative approaches.[30] During UTI many immunogenic and cellular responses determine the bacterial pathogenicity and other secondary manifestations. Consequently the virulence of bacteria infecting mucosal surfaces is thought to depend on the ability of the microorganism to adhere to epithelial tissue in the body.[31]

The results obtained in this study thus suggest the identified phytochemical compounds may be the bioactive constituents and these plants are proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merits.

CONCLUSIONS

Although polyherbal formulation is commonly used in many parts of the world, but the scientific evidence is still lacking. Many herbal therapies are still under in vivo evolution and have not been evaluated by clinical trials. There is need of time to evaluate polyherbal formulation using scientific method such as clinical trials, possible bioactive compounds and mechanism of action for the future world. Plant extracts have a great potential as antibacterial compounds against microorganisms. Thus they can be used in the treatment of infectious diseases caused by resistant microbes.

The result of this study reveals that both the aqueous and ethanolic extract were active against the strains of bacteria that are common cause of infections. The leaf extracts of polyherbal formulation shows significant effect that may be due to the presence of many potent compounds such as alkaloids, tannins, phenolic substances and glycosides etc. The antibacterial activity was expressed at varying degrees which was being both strains and dose dependent. The result of this study was found very encouraging and indicate that should be studied more extensively to explore its potential in the treatment of many infectious diseases.

REFERENCES

1. Subapriya, R., & Nagini, S. Medicinal properties of neem leaves: a review. Current Medicinal Chemistry-Anti-Cancer Agents, 2005; 5(2): 149-156.
2. Barman, P., Ahmed, N., & Chakraborty, D. Neem-a cynosure of modern medicine: A review. Int J Livest Res, 2019; 9: 1-7.
3. Koona, S., & Budida, S. Antibacterial Potential of the Extracts of the Leaves of Azadirachta indica Linn. Notulae Scientia Biologicae, 2011; 3(1): 65-69.
4. Uzzaman, S. Pharmacological activities of neem (Azadirachta indica): A review. Int J Pharmacogn Life Sci, 2020; 1: 38-41.
5. Bhowmik, D., Chiranjib, Y. J., Tripathi, K. K., & Kumar, K. S. Herbal remedies of Azadirachta indica and its medicinal application. J Chem Pharm Res, 2010; 2(1): 62-72.
6. Biswas, K., Chattopadhyay, I., Banerjee, R. K., & Bandyopadhyay, U. Biological activities and medicinal properties of neem (Azadirachta indica). Current science, 2002; 1336-1345.
7. Shareef, M., & Akhtar, M. S. Neem (Azadirachta indica) and its potential for safeguarding health, prevention and treatment of diseases. Matrix Science Medica, 2018; 2(1): 04-08.
8. Rahmani, A., Almatroudi, A., Alrumaihi, F., & Khan, A. Pharmacological and therapeutic potential of neem (Azadirachta indica). Pharmacognosy Reviews, 2018; 12(24).
9. Eid, A., Jaradat, N., & Elmarzugi, N. A Review of chemical constituents and traditional usage of Neem plant (Azadirachta Indica). Palestinian Medical and Pharmaceutical Journal (Pal. Med. Pharm. J.), 2017; 2(2): 75-81.
10. Bhattacharyya, K. G., & Sarma, A. Adsorption characteristics of the dye, Brilliant Green, on Neem leaf powder. Dyes and pigments, 2003; 57(3): 211-222.
11. Bhattacharyya, K. G., & Sharma, A. Azadirachta indica leaf powder as an effective biosorbent for dyes: a case study with aqueous Congo Red solutions. Journal of Environmental Management, 2004; 71(3): 217-229.
12. Chequer, F. M. D., Dorta, D. J., & de Oliveira, D. P. Azo dyes and their metabolites: does the discharge of the azo dye into water bodies represent human and ecological risks. Advances in treating textile effluent, 2011; 48: 28-48.
13. No, H. K., & Meyers, S. P. Crawfish chitosan as a coagulant in recovery of organic compounds from seafood processing streams. Journal of Agricultural and Food Chemistry, 1989; 37(3): 580-583.
14. Petrovska, B. B. Historical review of medicinal plants' usage. Pharmacognosy reviews, 2012; 6(11).
15. Ahvazi, M., Akbarzadeh, M., Khalighi-Sigaroodi, F., & Kohandel, A. Introduce some of the medicinal plants species with the most traditional usage in East Mazandaran Region. Journal of medicinal plants, 2012; 11(44): 164-175.

16. Joseph, S., & Peter, K. V. Curry leaf (Murraya koenigii), perennial, nutritious, leafy vegetable. Economic Botany, 1985; 39(1): 68-73.
17. Bhuyan, A. K., Pattnaik, S., & Chand, P. K. Micropropagation of curry leaf tree [Murraya koenigii (L.) Spreng.] by axillary proliferation using intact seedlings. Plant Cell Reports, 1997; 16(11): 779-782.
18. Parul, S., Javed, A., Neha, B., Honey, J., & Anuj, B. Curry leaves–A medicinal herb. Asian Journal of Pharmaceutical Research, 2012; 2(2): 51-53.
19. Rana, V. S., Juyal, J. P., & Blazquez, M. A. Chemical constituents of the volatile oil of Murraya koenigii leaves. International Journal of Aromatherapy, 2004; 14(1): 23.
20. Cline, R. J., & Haynes, K. M. Consumer health information seeking on the Internet: the state of the art. Health education research, 2001; 16(6): 671-692.
21. Vohora, S. B., Rizwan, M., & Khan, J. A. Medicinal uses of common Indian vegetables. Planta medica, 1973; 23(04): 381-393.
22. Malode, G. P., Parbat, A. Y., Shaikh, A. R., Panchale, W. A., Manwar, J. V., & Bakal, R. L. Phytochemistry, pharmacology and botanical aspects of Murraya Koenigii in the search for molecules with bioactive potential-A review. GSC Advanced Research and Reviews, 2021; 6(3): 143-155.
23. Kumar, A. B., Shamim, H., & Nagaraju, U. Premature graying of hair: review with updates. International Journal of Trichology, 2018; 10(5): 198.
24. Batool, S., Khera, R. A., Hanif, M. A., Ayub, M. A., & Memon, S. Curry leaf. In Medicinal Plants of South Asia, 2020; 179-190.
25. Hullatti, K. K., & Bhattacharjee, P. Pharmacognostical evaluation of different parts of Coleus amboinicus lour., Lamiaceae. Pharmacognosy Journal, 2011; 3(24): 39-44.
26. Kumaran, A., & Karunakaran, R. J. Activity-guided isolation and identification of free radical-scavenging components from an aqueous extract of Coleus aromaticus. Food Chemistry, 2007; 100(1): 356-361.
27. Romani, A., Pinelli, P., Mulinacci, N., Vincieri, F. F., & Tattini, M. Identification and quantitation of polyphenols in leaves of Myrtus communis L. Chromatographia, 1999; 49(1): 17-20.
28. Gurib-Fakim, A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine, 2006; 27(1): 1-93.
29. Balne, S., & Amutha, A. Development of a Healthy Noodles Enriched with the Flavors of Coleus Aromaticus, Mentha, Erythrina Indica. International Journal of Scientific Research & Engineering Trends, 2020; 6(6): 3265-3268.
30. Liao, Y. L., Chiang, Y. C., Tsai, T. F., Lee, R. F., Chan, Y. C., & Hsiao, C. H. Contact leukomelanosis induced by the leaves of Piper betle L.(Piperaceae): A clinical and histopathologic survey. Journal of The American Academy of Dermatology, 1999; 40(4): 583-589.
31. Hossain, M. F., Akhtar, S., & Anwar, M. Nutritional value and medicinal benefits of pineapple. International Journal of Nutrition and Food Sciences, 2015; 4(1): 84-88.