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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL EXPLORATION OF MEDICINAL PLANTS OF BALOCHISTAN AGAINST EYE INFECTION CONJUNCTIVES

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ABSTRACT

The present study was determined antimicrobial screening and phytochemical analysis of selected medicinal of Balochistan. This study creates an assessment on Conjunctivitis also known as "pink eye" in Balochistan province. Different antibiotics are used for the treatment of Conjunctivitis, but the Antibiotic resistance is a persistently emergent problem all over the world. So, the bioorganic chemical constituents of plant extracts play a vital role in antimicrobial drug discovery. The province of Balochistan contains a wide range of medicinal herbs, but still not evaluated technically. So, the different medicinal plants were tested against the bacterial strains which showed good antimicrobial activity. The crude methanol extracts (MCE) of three medicinal plants of Balochistan including *Achillea wilhelmsii C. Koch, Ferula oopoda, and Rhazya stricta Decne* were tested for a comprehensive selection of bacteria which cause conjunctivitis in areas of Balochistan namely *Staphylococcus aureus, Streptococcus pneumoniae*, *Haemophilus influenza* and *Pseudomonas aeruginosa*. All the medicinal plants extracts were found effective against the bacteria which cause eye conjunctivitis. *Rhazya stricta Decne* showed higher zone of inhibition, *Ferula oopoda* showed moderate while *Achillea wilhelmsii C. Koch* showed minute zone of inhibition. The results found in our current research might help the ethnobotanical significance of the screened medicinal plant species of Balochistan for the treatment of conjunctivitis.

Keywords: Phytochemical, Conjunctivitis, antibiotics, antimicrobial activity, *Achillea wilhelmsii C. Koch, Ferula oopoda, Rhazya stricta Decne*

INTRODUCTION

Conjunctivitis is the most common cause of the clinical problem, the red eye [1]. There are several other causes of the red eye including blepharitis, episcleritis, keratitis *etc.* [2] and various allergic clinical conditions are reported [3]. The effective management for such common condition might be achieved through a step-care approach, starting with identifying the allergen and avoiding the antigen [4] and sublingual immunotherapy is reported for the treatment of the allergic conjunctivitis [5]. Conjunctiva, which is the place where conjunctivitis occurs, provides a major source of immune components in the cornea. It produces the antigen immunoglobulin A that plays a critical role in mucosal immunity and also contains macrophages, neutrophilic granulocytes, mast cells, lymphocytes, and other aspects of the general mucosal immune system. The macrophages play a part in modulating the T-cell immune response and mediating both the innate and acquired immune responses [6].

Conjunctivitis is the inflammation of the conjunctiva due to various infectious and noninfectious agents. The bacterial conjunctivitis is represented by red eye [7]. Bacterial keratitis with potential complications is one of the most visually threatening ocular infectious pathologies. The corneal perforations have been reported in the presence of particularly invasive pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* [8]. Conjunctivitis can be caused by viruses, bacteria or fungi, exposure to chemicals or irritants or long-term presence of a foreign body such as hard or rigid contact lenses [9]. The traditional uses of the medicinal plants for the treatment of bacterial infections have, to minor extent, been investigated scientifically [10]. Due to the side effects exhibited by the conventional medicines, the natural products provide some alternative therapeutics [11]. Plants are exploited as medicinal source since ancient age. Medicinal plants have been established for millennia and are highly valued all over the world as a rich source of therapeutic agents for the prevention of diseases and ailments [12].

The traditional and folk medicinal system uses the plant products for the treatment of various infectious diseases [13]. Many edible plants are known to have beneficial and medicinal properties to humans, and plant extracts have been used as a source of alternative medication for their antibacterial, antifungal, and anticancer properties [14].

Plants have shown considerable activity against various microbes [15]. It is considered that plants are a source of a wide variety of bioactive molecules that can be used for the development of new medicines with a wider spectrum of activities and with less adverse effects than those produced by the drugs currently in use [16]. Bulgarian folk medicine treats conjunctivitis by several plants. Most popular are species of the genus *Euphrasia*, and even their common names are related to that use "ochanka" (in Bulgarian Ochi: eyes) [17]. There are empirical data for a therapeutic effect of *Geumurbanum*, *Althea officinalis*, *Pimpinela saxifraga*, *Anagalis arvensis*, in cases of conjunctivitis [18]. Members of the Crassulaceae family are known for their antiseptic and antibacterial properties. Particularly, leaves of *Echeveria gigantean* Rose and Purpus are used for eye illness treatment [19], but there are no data about the chemical or biological studies.

MATERIALS AND METHODS

Collections of Plants Materials

All the plant species were collected from different area of Balchiostan in June 2016 and identified substantiate by Prof. Dr. Ruksana Jabben, SBKWU. The plant samples washed and dried in shade.

Preparation of Extracts

The shade dried plants material converted to powder state by undergoing the grinding process by means of grinder. Each plant material was drenched for three days and extracted in methanol on Soxhelet apparatus discretely. Then, each filtrate was concentrated separately to a thick paste and desiccated under vacuum by using rotary evaporator**.**

Phytochemical screening:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Detection of alkaloids:

Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test:

Filtrates were treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colored precipitate indicates the presence of alkaloids.

Wagner's Test:

Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test:

Filtrates were treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test:

Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow colored precipitate.

1. **Detection of carbohydrates:**

Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Molisch's Test:

Filtrates were treated with 2 drops of alcoholic α-naphthol solution in a test tube. Formation of the violet ring at the junction indicates the presence of Carbohydrates.

Benedict's test:

Filtrates were treated with Benedict's reagent and heated gently. Orange red precipitate indicates the presence of reducing sugars.

Fehling's Test:

Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides:

Extracts were hydrolysed with dil. HCl, and then subjected to test for glycosides.

Modified Borntrager's Test:

Extracts were treated with Ferric Chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicates the presence of anthranol glycosides.

Legal's Test:

Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red color indicates the presence of cardiac glycosides.

5. Detection of saponins

Froth Test:

Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Foam Test:

0.5 gm. of extract was shaked in 2 ml of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

6. Detection of phytosterols

Salkowski's Test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few

drops of Conc. Sulphuric acid, shacked and allowed to stand. Appearance of golden yellow color indicates the presence of triterpenes.

Libermann Burchard's test:

Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. Sulphuric acid was added. Formation of brown ring at the junction indicates the presence of phytosterols.

7. Detection of phenols

Ferric Chloride Test:

Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

8. Detection of tannins

Gelatin Test:

To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

9. Detection of flavonoids

Alkaline Reagent Test:

Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicates the presence of flavonoids.

Lead acetate Test:

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates the presence of flavonoids.

10. Detection of proteins and amino acids

Xanthoproteic Test:

The extracts were treated with few drops of conc. Nitric acid. Formation of yellow color indicates the presence of proteins.

Ninhydrin Test:

To the extract, 0.25% w/v ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acid.

11. Detection of Diterpenes

Copper acetate Test:

Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green color indicates the presence of diterpenes.

Determination of Test Microorganisms:

Staphylococcus aureus, Streptococcus pneumoniae, *Haemophilus influenza* and *Pseudomonas auregenosa* bacteria were used for the study. These cultures were maintained on nutrient agar plates at 4°C.

Disc Diffusion Method

The extract from *Rhazya stricta Dcne, alownch, Achilliwismea, and Ferula oopoda* were investigated for antibacterial activity. For this reason, required measure of

supplement agar media and supplement stock was readied in flagons and was cleaned in autoclave. After cleansing, supplement agar media was filled the plates in a laminar stream hood and was brooded at 37°C for 24 hours to check any defilement. The microbial stock society was roused on supplement agar plates by streaking (known as first streak) with the assistance of a sterile immunization circle. The primary streak society was again streaked (known as second streak) on the crisp media plates and after that brooded at 37°C for 24 hours. The second streaked society was vaccinated into the sanitized supplement juices in jars which were then hatched in the shaking water shower for 18 hours at 37°C. The microbial societies from jar were institutionalized in cleaned supplement stock in the test tube. Institutionalized microbial inoculums were seeded into the supplement agar plates. Whatmann channel paper circles having 6 mm width were put on agar media and concentrates in various focuses were poured on the plates. Zone of hindrance was measured in mm and with the assistance of advanced camera photos of the antimicrobial movement were caught.

Statistical analysis

The data were analyzed statistically using SPSS. The strength of relationship between quantitative measures will be assessed using correlation coefficient. Chi-square test will also be applied to find association between qualitative attributes.

Table 1: Frequency of bacterial isolates in relation to age of patients

Organisms	0-2$^+$ N (%)	3-11$^+$N (%)	12-17$^+$N (%)	18-39$^+$N (%)	40 & above N (%)
Staphylococcus. aureus	40(28.80%)	33(25.50%)	20(22.70%)	20 (14.40%)	20 (14.40%)
Streptococcus pneumoniae	24(30.0%)	21(26.30%)	12(18.20%)	8(13.50%)	7(11.0%)
Haemophilus influenzae	8(10.20%)	6(6.80%)	6 (8.50%)	10(16.20%)	12(124.0%)
Pseudomonas aeruginosa	8(10.20%)	10(12.30%)	8(12.10%)	12(18.80%)	20(40.0%)

N = Number isolated

RESULT AND DISCUSSION

Hospital surveys:

The hospital survey of Bolan medical hospital and Helpers eye hospitalin Quetta city of Balochitan, revealed that The age groups 0- 2$^+$ and 3-11$^+$ recorded the highest cases of conjunctivitis 40 (28.80%) and 33(25.50%) of 208 respectively and Conjunctivitis was least 20 (14.40%) each in the 18-39$^+$ and in the 40s and above due to these bacteria. The table-2 showed the rate of isolation of different bacteria which cause eye conjunctivitis in the area of Balochistan according to survey in Helpers eye hospital is *S. aureus* cause highest rate of conjunctivitis. 46 % *S. pneumoniae* also cause conjunctivitis 29% and *H. influenza* is involved in eye infection 17.7% % the least

conjunctivitis is caused due to *P. aeruginosa* in different area of Balochistan show in Fig-10. The results tabulated in Table 3 determined the antibacterial activity of crude extracts of *Achillea wilhelmsii* C. Koch, *Ferula oopoda, and Rhazya stricta Decne* by means of both disk-diffusion and micro dilution techniques [20]. Current examination revealed the verification of antibacterial activity against some bacterial strains (*S. aureus, S. pneumoniea, H. influenzea, P. aeruginosa*) using plant extracts. The extracts of the *Rhazya stricta Decne* plants shown highest antibacterial activity while the extracts of the *Ferula oopoda* showed moderate antibacterial activity and *Achillea wilhelmsii* showed lowest antibacterial activity against *S. aureus, S. pneumoniea, H. influenzea, P aeruginosa* (Fig-11).Standardized antibiotics are used as positive control (Ciprofloxicin). Likewise, DMSO used as a negative controller does not showed any inhibition of bacterial growth**.**

The medicinal plants were collected, dried, crushed and subjected to extraction with (CH_3OH) methanol and this crude extracts were analysed for phytochemical analysis. Phytochemical test results showed in Table-4 proved the existence of steroids, flavonoids, tannins, carbohydrates, saponins, and fixed fats and oils presence in selected medicinal plant extracts.

Table 2: Rate of Isolated Bacteria

Organisms	Percentage
S. aureus	**46%**
S. pneumoniae	**29%**
H. influenzae	**17.70%**
***P.* aeruginosa**	**8%**

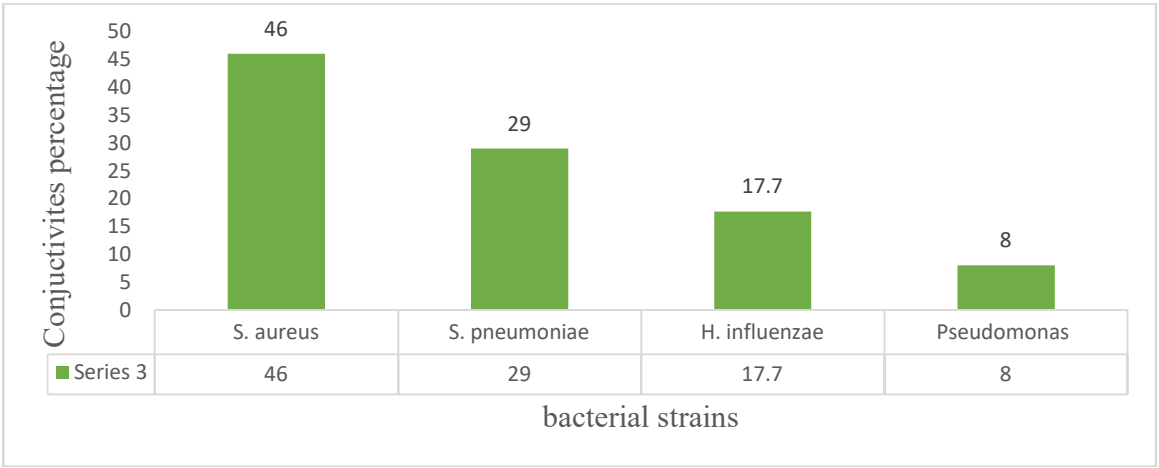

Fig 1: Rate of conjunctivitis vs. bacterial strain

Table 3: Inhibitory zone of the different extracts against four bacterial strains

Bacterial strains	Plants Extract	Inhibition Zone	Standard (ciprofloxacin)	DMSO
S. aureus	*Rhazya stricta Decne*	14	11.5±0.71	NA
S. pneumoniae	*Rhazya stricta Decne*	13	11.75±1.1	NA
H. influenza	*Rhazya stricta Decne*	13	14.25±1.1	NA
P. aeruginosa	*Rhazya stricta Decne*	11	19.25±1.1	NA
S. aureus	*Ferula oopoda*	11	11.5±0.71	NA
S. pneumonia	*Ferula oopoda*	13	11.75±1.1	NA
H. influenza	*Ferula oopoda*	12	14.25±1.1	NA
P. aeruginosa	*Ferula oopoda*	13	19.25±1.1	NA
S. aureus	*Achillea wilhelmsii*	12	11.5±0.71	NA
S. pneumonia	*Achillea wilhelmsii*	9	11.75±1.1	NA
H. influenza	*Achillea wilhelmsii*	11	14.25±1.1	NA
P. aeruginosa	*Achillea wilhelmsii*	9	19.25±1.1	NA

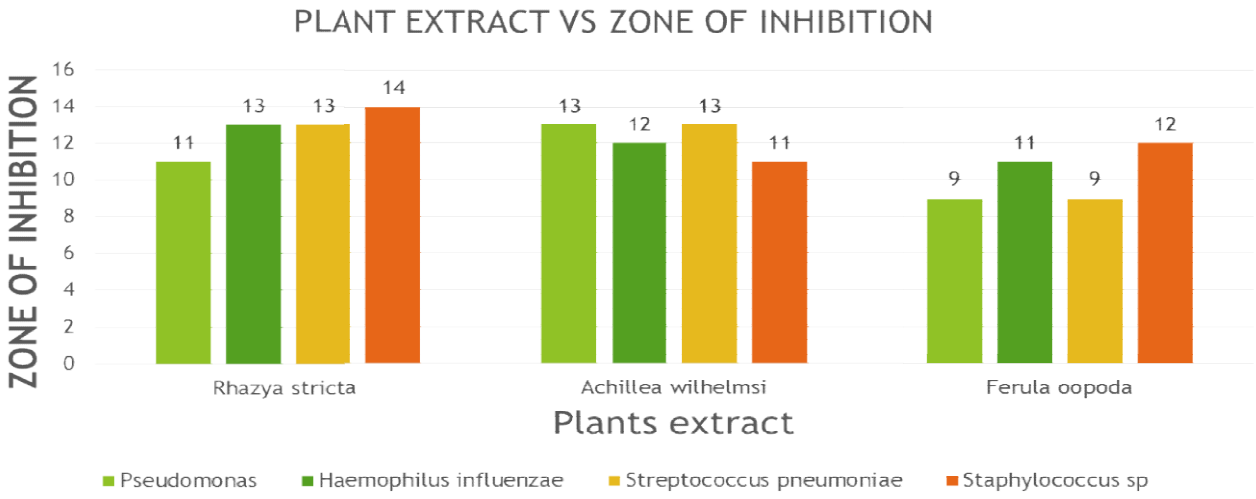

Fig 2: Plants Extract Vs. zone of inhibition

Table 4: Phytochemical Screening Test

Medicinal Plants extracts			
Plants constituens	***Ferula oopoda***	***Achillea wilhelmsii***	***Rhazya.stricta* Dcne**
Terpenoids	+	+	+
Flavonoids	+	+	+
Alkaloid	+	+	+
Carbohydrates	+	_	_
oils and Fats	+	+	+
Glycosides	+	_	_
Phenol	+	+	+
Tannins	+	+	+
Steroid	+	_	_
Proteins	+	+	_
Saponins	_	+	+

CONCLUSION

Achillea wilhelmsii C.Koch, *Ferula oopoda,* and *Rhazya stricta Decne* distributed in different area of Balochistan have an important medicine plants. The present study was conducted on qualitative and quantitative phytochemical analysis, and *in vitro* biological activity such as antibacterial activity. The methanol extracts of *Achillea*

wilhelmsii C.Koch, *Ferula oopoda,* and *Rhazya stricta Decne* have different types of phytochemicals which might be responsible for antimicrobial activity. The presence of phytochemicals might be responsible for their therapeutic effects. All the plants were verified for a comprehensive selection of bacteria which cause conjunctivitis in area of Balochistan (*Staphylococcus aureus, Streptococcus pneumoniae*, *Haemophilus influenza* and *Pseudomonas auregenosa).* All the medicinal plants extracts were effective against the bacteria which cause eye conjunctivitis. *Rhazya stricta Decne* showed higher zone of inhibition*, Ferula oopoda* showed moderate while *Achillea wilhelmsii C. Koch* showed minute zone of inhibition. It further reflects a hope for the development of many novel chemotherapeutic agents or templates from such plants which in future may serve for the production of synthetically improved therapeutic agents.

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