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Standardization and Evaluation of Polyherbal Syrup are Containing *Aconitum Hetrophyllum* and *Caesalpinia Bonducella* for its Antimicrobial Activity.

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Abstract

The Anti-microbial activity of ethanolic extract of *Aconitum hetrophyllum and Caesalpinia bonducella* has been evaluated. Then poly herbal syrup by using both extracts has been prepared and again antimicrobial activity of polyherbal syrup has been checked by using agar plate diffusion method. The result demonstrates the antimicrobial activity of both the extract. Antimicrobial effect was compared to poyherbal syrup, which shows synergistic effect. It sounds like the research evaluated the antimicrobial activity of the ethanolic extract of *Aconitum hetrophyllum* and *Caesalpinia bonducella* individually, and then combined them to create a polyherbal syrup, which also showed antimicrobial activity. The agar plate diffusion method was used to assess the antimicrobial effects. The results indicated positive antimicrobial activity for both the extracts and the polyherbal syrup.

Keywords: Polyherbal syrup, Formulation, Antimicrobial activity, gastrointestinal infection

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1. Introduction

The majority of old medical systems are efficient, yet there is a lack of sufficient standards. Therefore, WHO mentioned the specific guidelines for assessment of identity, quality, safety, and efficacy of herbal drugs and formulations, Standardization is important for quality assurance of any formulation, and its process in which physiochemical evaluation of crude drugs, safety, and effectiveness is occurs [1]. Polyherbal syrup were prepared by using different herbal or crude drug extracts of *Aconitum hetrophyllum, Caesalpinia Bonducella* for antimicrobial activity by exploiting knowledge of traditional system of medicine [2]. These studies were conducted that standardization and evaluation of polyherbal syrup for its antimicrobial activity Plant based crude drug are very effective which are used in modern pharmacy to maintain safety, quality and efficacy of plant and their product is essential to avoid serious health issues. Some standard solutions or preparations are used for comparison of test solution1. The current study focused on polyherbal syrup, a mixture of *Aconitum hetrophyllum and Caesalpinia bonducella*, for its antibacterial properties. Quaternary and diterpene alkaloids are two prevalent phytoconstituents that have been shown to have antibacterial action. There are numerous sorts of infections brought on by bacteria, and polyherbal antimicrobial syrup is used to treat these infectious disorders. The ingredients in syrup—such as Entoban syrup, a mixture of *Holarrhena antidysenterica,*

Berberi saristata, Symplocos racemosa, Querecus infectoria, and Helicte resisora—eradicate microorganisms from the digestive system [3, 4, 5].

Gallic acid, a common phytonutrient included in Entoban syrup, has been shown to have antioxidant and antimicrobial properties. Polyherbal syrup should be evaluated and standardized to ensure its potency and quality. Currently available herbal antibacterial preparations do not specifically target gastrointestinal tract infections. There are many products from different businesses that have formulations for syrup, avaleha, arishta, asava, malam, and ghrit, among others. Aconitum hetrophyllum and Caesalpinia bonducella are two medications with antibacterial action against several microorganisms that cause gastrointestinal tract infections [6, 7, 8]. The goal of the current endeavor is to create antibacterial preparations employing both herbal and ayurvedic plant materials. To uniformize and assess this preparation using pharmacognostic methods.

2. Materials and Methods

2.1 Plant Profile:

***Aconitum hetrophyllum*:**

Title: - *Aconitum hetrophyllum*

Biological Source**:** - Ativisha consist of dried tuberous roots of *Aconitum hetrophyllum* Wall. Ex. royale

Family: - *Ranunculaceae* Synonym: - Ativish, Atish Chemical Constituent : -

The Medicinal plant of *Aconitum hetrophyllum* is rich source of Alkaloids. The chief constituent is diterpene alkaloids (0.7%) The alkaloids mainly contain diterpene alkaloid such as atisine (I) and atidine (II) and aconitine, atesine, dihydroatisine hetratesine, hetrophyline, hetrophyllisine, hetrophylidine also hetisined and heraitsine. Other constituents are carbohydrate, proteins & amino acid, saponine, quinones, flavonoids, polysaccharides and free fatty acids etc. Many of which alkaloids gives broad spectrum activity, data given in table 1.

Medicinal Uses:

Aconitum hetrophyllum has lots of medicinal value or uses. The juice of root with milk acts as expectorant, it gives antidiarrheal effect with other herbs like ginger, nutmeg, bael patra. It's also used as analgesic, antipyretic anti-inflammatory, anti-flatulent, and antiperiodic etc. The diterpene alkaloids gives cardiac effect with antiarrhythmic activity, analgesic effect and mainly antimicrobial effect. It's effective against gram negative and gram-positive bacteria. Also, it acts as anthelmintic, emetic and antidysentric [16].

Caesalpinia bonducella:

Title: - *Cisalpenia Bonducella*

Biological Source: -

It consists of dried seeds of *Caesalpinia bonducella* (Linn) Roxb

Family: - Fabaceae/ Cisalpiniaceae

Synonym: - Sagargota, Gaajagaa Chemical Constituent : -

Whole plant of *Caesalpinia Bonducella* contains major chemical constituent. These are Steroidal Saponin, Isoflavones, Phytosterol, fatty acids, Hydrocarbon, Phenolics and amino acids etc. Chemical constituents present in different part of plant like seed, seed kernels, leaves bark etc. Leaves contains calcium, glucose, pinitol, bark contains homoisoflavonoids. O-methyl Caesalpinianone and Caesalpinianone, Seed have been found to contain neutral Saponin, Furan diterpenes, Phytosterinin, beta-caesalpin, alpha Caesalpin, etc. Root founds to be contained Cassane furan diterpenes, caesalpinin, bonducellpins A, B, C, and D, [17, 18].

Medicinal Uses:

It has numerous therapeutic properties. *Caesalpinia bonducella* is widely employed in the traditional form of Indian medicine known as Ayurveda for its range of therapeutic effects, including antipyretic, antiperiodic, anti-inflammatory, anthelmintic, and antimalarial properties. Escherichia coli, *Klebsiella aerogenes*, *Aspergillus niger*, and *Penicillium chrysogenum* are just a few of the viral and bacterial species that leaves extract has been shown to have antibiotic activity against. The stems indicate antiviral, neuroprotective, diuretic, and antifungal action in addition to other properties [19].

2.2 Important bacterial and viral pathogen which causes GIT infection.

There are various bacteria, virus, fungi which causes gastrointestinal infection are from animal water and from food sources. T44he important bacterial and viral pathogen which are from water food and animal sources mentioned in table given bellow [9, 10, 11].

Collection and authentication of plant material: Caesalpinia bonducella plant was collected from the local area and authenticated by the botany department of Y.C. Science College, Karad. Aconitum hetrophyllum roots and seeds were purchased from Dorle Ayurved Kolhapur. Selection and Evaluation of Herbs: All herbs were selected and shade dried, then coarsely powdered and tested for its macro and microscopic characters and physiochemical properties [12, 13].

2.3 Standardization Parameters:

Standardization parameters are studied by considering the various point according to the WHO guidelines. It's followed by colour odour taste LOD, total ash, sulphated ash, different extractive values given in table no. 2, 4.

2.4 Preparation extract of roots Aconitum hetrophyllum:

The methanolic extract of the roots of Aconitum hetrophyllum was made in accordance with the standard extraction method previously published (Trease and Evans) [14]. The gathered roots were first dried at room temperature in the shade before being ground into a coarse powder. After that, 80% ethanol was continuously extracted from dry powder using a Soxhlet equipment. Whatmann's filter paper was used to pack the powdered root material. It underwent 40 rounds of ethanol extraction. The extract was obtained, and it was then condensed to dryness in a flash evaporator at a controlled temperature and low pressure. The yield of methanolic extract was a thick, sticky paste with a yellowish brown

color. It was kept in the fridge [15]. Phytochemical evaluation done and given table 5 and 6.

2.5 Preparation extract seeds of Caesalpinia Bonducella:

The Caesalpinia Bonducella methanolic extract was made using the previously reported standard extraction method (Trease and Evans).

The gathered seeds were first dried at room temperature in the shade before being ground into a coarse powder. After that, 80% ethanol was continuously extracted from dry powder using a Soxhlet equipment. Whatmann's filter paper was used to pack the powdered root material. It underwent 40 rounds of methanol extraction. The extract was obtained, and it was then condensed to dryness in a flash evaporator at a controlled temperature and low pressure [20, 21]. Phytochemical evaluation done and given table 6.

The result of ethanolic extract was a thick, sticky paste with a brown color. It was kept in the fridge.

Simple Syrup Preparation Method: Purified water was added to 666.7 g of weighed sucrose, which was then heated until it dissolved while being occasionally stirred. 1000ml of hot water was supplied in total, [22].

2.6. Final Syrup Preparation Procedure:

Using the Soxhlet apparatus and the ethanol (80%) as the solvent, extracts of the plants Caesalpinia bonducella and Aconitum hetrophyllum were made.

Final polyherbal syrup prepared using the accepted procedure. Since simple syrup has a built-in preservative, only one part of extract was combined with five parts of the sweetener. The resultant herbal syrup was then put to a test [23, 24, 25].

2.7 Evaluation of Antimicrobial Activity of Final Prepared Syrup:

The goal of the current investigation was to assess the antibacterial efficacy of a polyherbal formulation against microorganisms commonly associated with gastrointestinal illnesses. A synergistic effect was also explored. By using the agar well diffusion method, the antimicrobial activity of Taphylococcus aureus was assessed against five gram negative bacterial cultures, including Salmonella enteric, Eschericia coli, Shigella dysenteriae, Pseudomonas aeruginosa, and Vibrio cholera. These organisms' development was restricted by the prepared syrup. According to our research, poly herbal preparations have a strong potential to combat pathogenic bacteria and can be utilized as an antimicrobial agent to treat a variety of infectious disorders of the gastro intestinal tract [26, 27, 28].

2.8 Test Microorganism:

The microbiology lab provided the testing organism. Staphylococcus aureus and Bacillus subtilis, two gram positive bacteria, and Escherichia coli, a gram negative bacteria, were used in the experiment. The nutrient agar and saline solution were autoclave sterilized. Before use, all equipment was sterilized in an oven. For 24 hours, the fresh culture was incubated at 370 ˚C [29, 30].

2.9 Evaluation of Antimicrobial Activity: Agar Well diffusion method:

Using different bacterial culture strains, the agar well diffusion method was used to assess antimicrobial activity. We employed strains of Shigella dysentery, Escheria coli, Vibrio cholera, and Staphylococcus aureus [22].

2.9.1 Antimicrobial Activity Assay:

Evaluation of Aconitum hetrophyllum extract:

Using the agar well diffusion method, the antibacterial activity assay was evaluated. The following technique was carried out using ready-made agar plates. Using a sterile micropipette, 100 mL of *aconitum hetrophyllum extract* were applied to the wells. The plates were incubated for 24 hours at 370C. By assessing the surrounding well's zone of growth inhibition, the antibacterial activity was assessed [23]. By using Vernier callipers, the diameter of the inhibitory zone was determined in millimeters (mm) as shown in figure 2.

2.9.2. Caesalpinia bonducella extract evaluation:

Using the agar well diffusion method, the antibacterial activity assay was evaluated. The following technique was carried out using ready-made agar plates. Using a sterile micropipette, the 100 mL of *Caesalpinia bonducella* extract was applied to the wells. The dinnerware was incubated for 24 hours at 370C. By assessing the surrounding well's zone of growth inhibition, the antibacterial activity was assessed [24]. By using Vernier callipers, the diameter of the inhibitory zone was determined in millimeters (mm) shown in figure 3.

2.10 Evaluation of Final Preparation of Syrup:

Using the agar well diffusion method, the antibacterial activity assay was evaluated. The following technique was carried out using ready-made agar plates. Using a sterile micropipette, the test solution of the final syrup preparation, 5ml, was put into the wells. For 24 hours, the plates were incubated at 370C. By assessing the surrounding well's zone of growth inhibition, the antibacterial activity was assessed. By using Vernier callipers, the diameter of the inhibitory zone was determined in millimeters (mm) [31, 32, 33].

Concentration of Aconitum hetrophyllum root extract and Caesalpinia bonducella seed extract *A-Escheria Coli B-Staphylococcus aureus C Vibrio cholare D Shigella* dysentery shown in table 8 [34,35].

3. Results and discussion

Diterpenoid and quaternary alkaloids can be found in the plants *Aconitum hetrophyllum and Caesalpinia bonducella*. By using the agar well diffusion method, methanolic extracts of the roots of *Aconitum hetrophyllum and the seeds of Caesalpinia bonducella* demonstrated antibacterial efficacy against gastrointestinal infection-causing bacteria like Staphylococcus aureus, E. coli, Vibrio cholare, and Shigella dysentery. As a result, this plant can be utilized to treat gastrointestinal infections in people. So, employing both plants, I created an antibacterial poly herbal mixture. The end result demonstrates *that E. coli, Staphylococcus aureus, Vibrio cholerae, and Shigella*

dysentery are all bacteria that can cause gastrointestinal infections, and both the medications *Aconitum hetrophyllum and Caesalpinia bonducella* extract independently exhibit antimicrobial action against these bacteria. By measuring the zone of inhibition, the agar plate method was used to study its antibacterial activity shown in table 9. The polyherbal syrup formulation, which includes *Aconitum hetrophyllum and Caesalpinia bonducella*, is the finished herbal syrup preparation and exhibits antibacterial activity against bacteria that cause gastrointestinal infections. This polyherbal syrup's activity goes beyond that of any medication extract individually. Consequently, it demonstrates that the produced polyherbal syrup composition has a synergistic antibacterial action.

Table 1: Numerous therapeutic properties

Sr.No.	Pathogen	Animal reservoir	Food borne	Water borne
	Bacteria			
1	Escheria Coli	+	+	+
2	*Staphylococcus aureus*	-	+	+
3	*Campylobacter*	+	+	+
4	*Shigella*	-	+	-
5	*Salmonella*	+	+	+
6	*Vibrio cholerae*	-	+	+
7	*Bacillus cereus*	-	+	-
8	*Yersinia enterocolictica*	+	+	-
9	*Vibrio parahaemolyticus*	-	+	+
	Viruses			
1	*Small round virus*	-	+	+
2	*Rotavirus*	-	-	-

Table 2: Standardization of root powder of *Aconitum hetrophyllum*

Sr No.	Parameters	Physiochemical properties (%w/w)
1	Colour	Yellowish
2	Odour	Pungent
3	Taste	Bitter
4	Total Ash	2.07
5	Acid insoluble ash	0.45
6	Water soluble ash	0.91
7	Foreign matter	Nil
8	Extractive value in alcohol	4.08
9	Extractive value in water	22.65

Table 3: Phytochemical screening of Aconitum hetrophyllum (+) = Presence (-) = Absence.

Sr.No.	Chemical Constituent	Phytochemical Test	Root
1	Alkaloids	Mayer's Test	**+**
2	Glycosides	Bromine Water Test	+
3	Flavonoids	----------	+
4	Terpenoids	----------	-
5	Saponin	Salkowask'y Test	+
6	Carbohydrate	Felhing's Test	+
7	Quinones	----------	+
8	Protein and Amino Acids	Ninhydrine Test	+

Table 4: Standardization of seed Powder of *Caesalpinia Bonducella*

Sr No.	Parameters	Physiochemical properties(%w/w)
1	Colour	Light brown
2	Odour	Characteristic
3	Taste	Astringent
4	Total Ash	3.34
5	Acid insoluble ash	0.42
6	Water soluble ash	1.61
7	Foreign matter	0.969
8	Extractive value in alcohol	2.73
9	Extractive value in water	6.4

Table 5: Phytochemical screening of *Caesalpinia bonducella* (+) = Presence (-) = Absence

Sr.No.	Chemical Constituent	Phytochemical Test	Seeds	Leaves
1	Alkaloids	Dragaendroff's Test	+	+
2	Tannin	Kokate's Method	++	+
3	Flavonoids	----------	+	++
4	Phenolic compounds	----------	+	+
5	Saponin	Salkowask'y Test	+	-
6	Carbohydrate	Felhing's Test	-	++
7	Quinine	----------	-	++
8	Protein and Amino Acids	Ninhydrine Test	+	++
9	Terpenoids	----------	+	+
10	Steroids	Salkowski Test	+	

Table 6: Phytochemical Screening of both.

Sr.No.	Chemical Constituent	Phytochemical Test	*Aconitum hetrophyllum*	Caesalpinia bonducella
1	Alkaloids	--Dragaendroff's Test	+	+
		-- Hager's Test	+	+
		-- Wagner's test	+	+
		-- Mayer's Test	-	-
2	Flavonoids	-- Shinoda's Test	+	+
3	Triterpenoids	-- Liebermann –Burchard's Test	+	+
4	Saponin	Salkowask'y Test	+	+
5	Carbohydrate	-- Anthrone Test	+	+
		-- Benedict's Test	+	+
		-- Felhing's Test	+	+
		--- Molisch's Test	-	+
6	Protein	-- Biuret's Test	+	+
		-- Millon's Test	+	+
7	Resin	Test for resin	+	+
8	Saponin	Test for Saponin	+	-
9	Tannins	Test for Tannins	+	+
10	Starch	Test for starch	+	-
11	Glycosides	Test for Glycosides	+	+

Table 7: Formula for polyherbal syrup

Name of Ingredient	Quantity taken

Aconitum Hetrophyllum extract	5g
Caesalpinia Bonducella extract	5g
Simple Syrup	50ml
Flavouring Agent	Q.S.

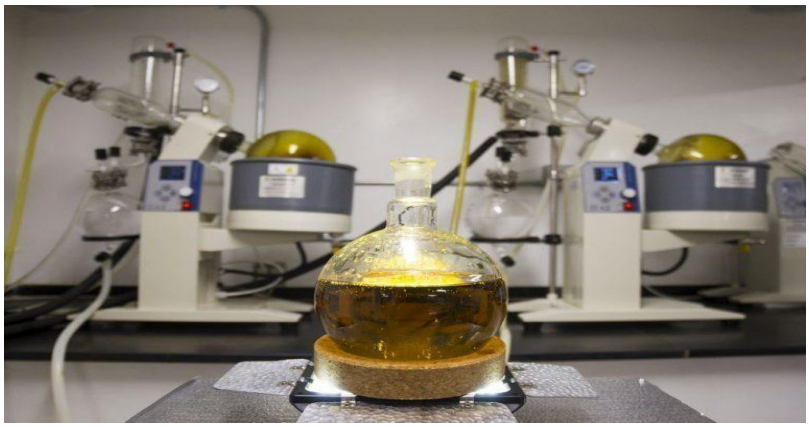
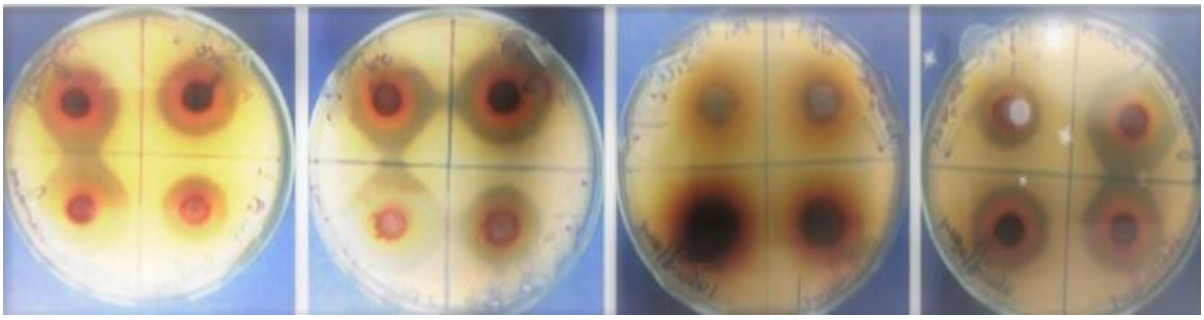


Figure 1: Photograph of extraction Assembly



A B C D

Figure 2. A- *Aconitum Hetrophyllum* B- *E. coli, Staphylococcus aureus, and C- Bacillus cholare D Shigella dysenteritis*

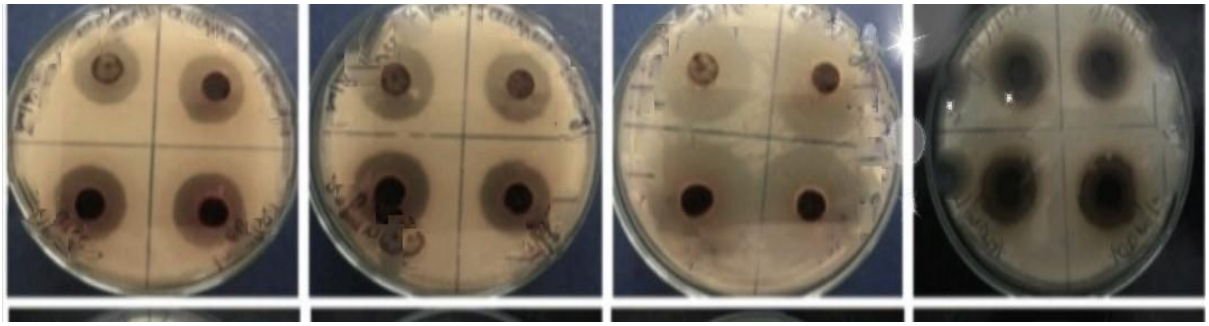


Figure 3. *A- Escheria Coli, B- Staphylococcus aureus, C- Vibrio cholare, D- Shigella dysentery*

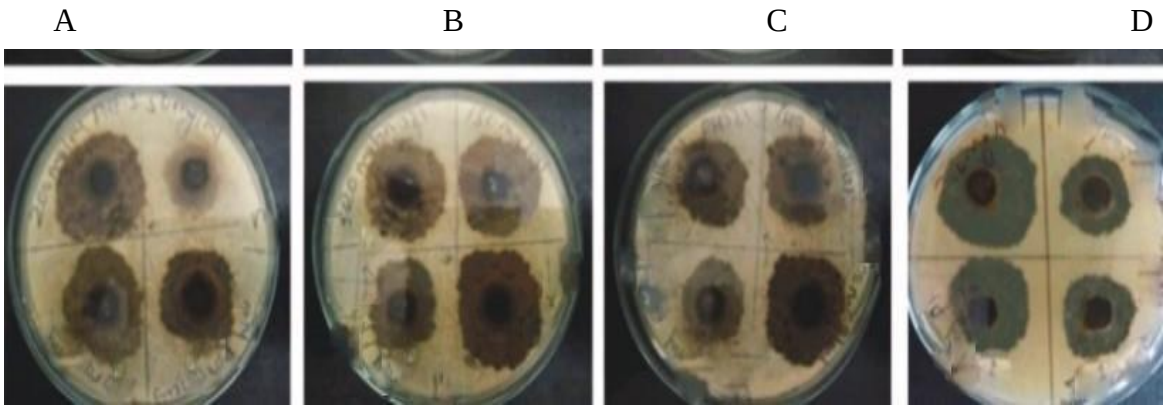


Figure 4. A-*Escheria Coli* **B**-*Staphylococcus aureus* **C** *Vibrio cholare* **D** *Shigella dysentery*

Table 8. MIC of Both Extract

Sr.no.	Concentration of Extract (5 ml)	Zone of inhibition (mm in diameter)			
		Escheria coli	***Staphylococcus aureus***	***Vibrio cholare***	***Shigella dysentery***
1	***Aconitum Hetrophyllum***	9	10.5	9	10
		10	11	12	11
		10	12	12	11
		10	12	12	12
2	***Caesalpinia bonducella***	9	9	6.5	9.5
		10.5	10	7	10.5
		10	10	7	10
		10	10	7.5	10

Table 9. MIC of Final poly herbal syrup

Sr.no.	Concentration Of Extract (5 ml)	Zone of inhibition (mm in diameter)			
		Escheria Coli	***Staphylococcus aureus***	***Vibrio cholare***	***Shigella dysentery***
1	Final syrup Preparation	11	12	12.5	13
		13.5	13	13	13.5
		13	13.5	13.5	13.5
		14	14	14	14.5

4. Conclusions

In comparison to individual drug extract, the final polyherbal syrup has a larger zone of inhibition. So, when used together, these two medications have a synergistic antibacterial action.

Conflict of interest

The authors declare no conflict of interest.

Author's contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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