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PHYSICOCHEMICAL AND BIOLOGICAL EVALUATION OF RAKTASHODHAKARISHTA

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ABSTRACT

The most well-known traditional Indian medical practices, such as Ayurveda, Sidha, Unani, and Homeopathy are gaining popularity across the globe. In the ayurvedic medical system, Arista is a self-made herbal mixture. Raktashodhakarishta is a classic Ayurvedic composition used for the skin diseases and blood purification. The aim of the present study is to identify the chemical components present in the Arista, such as alkaloids, phenolic and tannins, flavonoids, glycosides, carbohydrates, steroids, saponins, terpenoids and protein and amino acids. And to determine how they related to the anti-inflammatory, anti-bacterial, and anti-oxidant qualities. Also, the present study aims to determine the physicochemical properties like alcohol content, ash values, extractive values, moisture content, viscosity, and specific gravity. The anti-bacterial activity was evaluated by gram positive and gram-negative bacteria by agar plate method. The DPPH scavenging assay was used to assess the anti-oxidant activity. And anti-inflammatory property of the Arista was evaluated by the protein denaturation method by egg's albumin with using standard as Diclofenac Sodium.

KEYWORDS: Raktashodhakarishta, anti-inflammatory, anti-oxidant, anti-bacterial activity, DPPH.

INTRODUCTION

Ayurveda is an Indian traditional medicine being used for thousands of years. Ayurveda means, ' the science of life' in Sanskrit. Rather than treating illness, the goals of ayurvedic medicine is to promote health. Arista are traditional Ayurvedic self-herbal fermentation. These are alcoholic medications that are made by fermenting herbal liquids or decoction with the addition of sugars. They have pleasant perfume, a little acidity, sweetness, and amount of alcohol (up to 12 percent by volume). The presence of alcohol in the preparation has a number of benefits, including improving medicinal qualities, better preservation quality, more effective drug molecule extraction from the herbs, and more effective drug transport to human body sites. Raktashodhakarishta is a traditional herbal formulation having blood purifying properties. It is derived from the Sanskrit word. Main ingredients present in the Raktashodhakarishta are Sariva, Munakka, Kachnar, Khadira, Shirish, Indravaruni, Manjistha, Shatavari, Neem, and Dhataki flower etc. They show blood purifying properties. The quantitative and qualitative study of the Raktashodhakarishta guarantee the quality and the safety of the product. So, the determination of alcohol content, pH like physicochemical properties and phytochemical are highly significant. Anti-microbial properties of Ayurvedic herbs and formulations essential to determine. Many ingredients present in the Raktashodhakarishta produce anti-microbial activity. Anti-bacterial studies using gram positive and gram-negative bacteria. Many ingredients produce anti-inflammatory activity. It is determined by the invitro anti-inflammatory study by protein denaturation method by eggs albumin. Anti-inflammatory agents are used to relieve pain and inflammation, and regulate the body temperature. Antioxidants are the compounds, they will inhibit the oxidation (by chemical reaction, producing free radicals). In the Raktashodhakarishta determining the antioxidant activity by DPPH radical scavenging assay.[1][2][3][4]

MATERIALS AND METHODS

Organoleptic characters

Various parameters such as colour, taste and odour of the Arishta were observed and recorded.[5][6]

Physicochemical evaluation

In physicochemical screening, the Arishta sample were analysed for various physicochemical parameters such as alcohol content, extractive values, ash values, weight per ml, specific gravity, viscosity, relative density, total solid content, moisture content and pH.[5][6]

Phytochemical screening

In phytochemical screening, the Arishta sample were analysed for various phytochemical screening such as alkaloids, phenolic and tannins, flavonoids, saponins, proteins and amino acids, glycosides, terpenoids, steroids, and carbohydrates.[7]

Antimicrobial activity

In the determination of anti-microbial were evaluated by the agar well diffusion method. It is the commonly used method. For the evaluation of the anti-microbial activity, to prepare nutrient agar plates (Muller Hinton). For this, dissolve 1.52g of the dehydrated Muller Hinton agar medium in 40ml of the distilled water. Boil and dissolve, then sterilized by autoclaving at 121^{o}C for 15 minutes. Then inoculate the agar plates with bacterial culture of gram positive as well as gram negative bacteria. And creating the wells using the sterile borer or pipette tip. Using micropipette add Arishta and standard substance (Ciprofloxacin 200mg/100ml) to the separate wells. For 24 hours, plates were incubated at 37^{0}C. After incubation period observed the inhibition zone.[8]

Antioxidant activity

DPPH (2,2- diphenyl-1-picrylhydrazyl) Radical Scavenging Assay[9]

Using the stable DPPH radical, which has an absorption maximum at 515 nm, the total free radical scavenging capability of Arishta was calculated using the previously reported method with a minor modification. To make a solution of the radical, dissolve 2.4 mg of DPPH in 100 ml of Methanol. 3.995 ml of methanolic DPPH was mixed with 5µl of the test solution. The mixture was vigorously mixed and left at room temperature in the dark for 30 minutes. The reaction mixture's absorbance was spectrophotometrically measured at 515 nm. DPPH radical absorption in the absence of an anti-oxidant.

$$DPPH\ Scavenged\ (\%) = [(AB - AA)/AB \times 100$$

AB=Absorbance of blank control, AA=Absorbance of anti-oxidant

Anti-inflammatory activity

The evaluation of the anti-inflammatory activity by protein denaturation method using egg's albumin. To evaluating the anti-inflammatory, preparing the Phosphate buffer pH 7.4. activity is evaluated in the various concentrations of test solution (Arishta), standard (Diclofenac Sodium) and control. The reaction mixture (5ml) consists of 0.2 ml of egg's albumin, 2.8 ml of Phosphate buffer (7.4), and 2ml of varying concentrations (200,400,600,800, and 1000

µg/ml) of the tests or standard solution. Control containing 0.2ml of egg's albumin, 2.8ml of Phosphate buffer, and distilled water up to 5ml. All the solution were incubated at 37°C for 15 minutes. Then extend to 70°C for 5 minutes. After the incubation period, it was measured at the 280nm. The percentage inhibition calculated by,[10]

$$\%inhibition = \frac{Absorbance\ of\ control - Absorbance\ of\ test}{Absorbance\ of\ control} \times 100$$

Figure 1: Various concentrations of Diclofenac.

Figure 2: Various concentrations of Arishta.

RESULTS

Organoleptic characteristic

Table 1: Organoleptic characters.

Colour	Brown
Odour	Alcoholic, aromatic
Taste	Palatable in taste

Physicochemical screening

Table 2: Results of physicochemical evaluation.

SL.NO	PARAMETERS	RESULT
1	Total ash	2.6%w/w
2	Acid insoluble ash	1.14%w/w
3	Water soluble ash	1.5%w/w
4	Water soluble extractive value	2.56%w/w
5	Alcohol soluble extractive value	47.26%w/w
6	Viscosity	0.03 poise
7	Specific gravity	1.06g/ml
8	Total solid content	6,85%w/w
9	Weight per ml	2.26g/ml
10	Relative density	1.06g/ml
11	Moisture content	19.28g
12	Alcohol content	8%v/v
13	pH	4.1

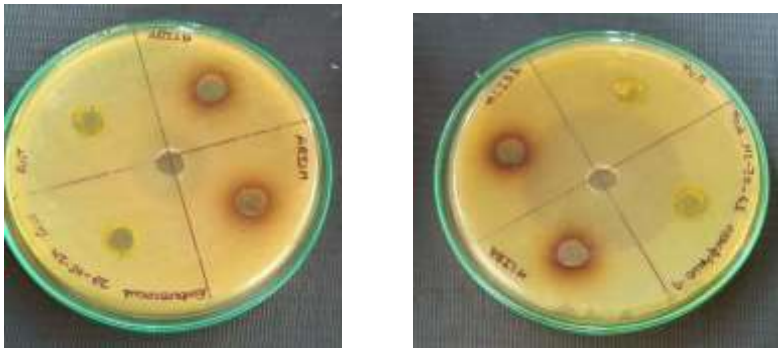
Phytochemical screening

Table 3: Results of phytochemical screening.

SL.NO	TESTS	RESULT
1	Alkaloids	+
2	Phenolics and tannins	+
3	Saponins	+
4	Flavonoids	+
5	Carbohydrates	+
6	Glycosides	+
7	Protein and amino acids	+
8	Terpenoids	+
9	Steroids	+

Antimicrobial Activity

Bacterial growth shown in the performed experiment for determination of anti-microbial activity of Raktashodhakarishta by agar well diffusion method.



Gram +ve: Pseudomonas aeruginosa **Gram -ve: Enterobacter**

Figure 3: Result of anti-microbial activity of standard and Arishta.

Antioxidant activity

Anti-oxidant activity shown in the performed experiment for determination of anti-oxidant activity of Raktashodhakarishta by method DPPH(2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay.

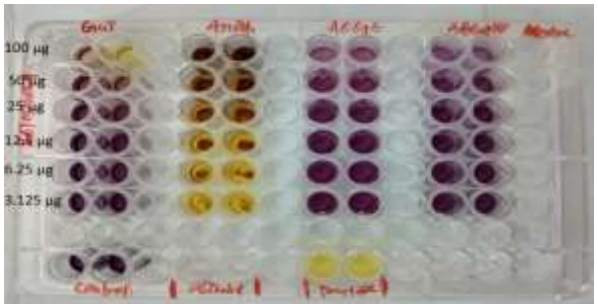


Figure 4: Result of Anti-oxidant activity by DPPH scavenging assay.

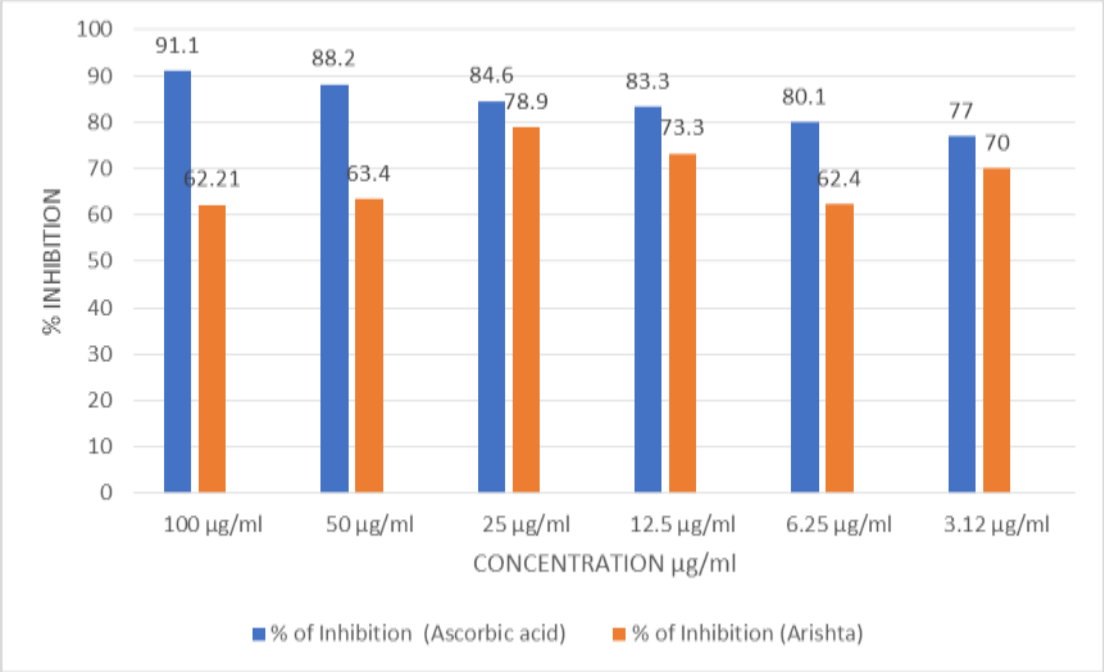


Figure 5: Graphical representation of % inhibition effect.

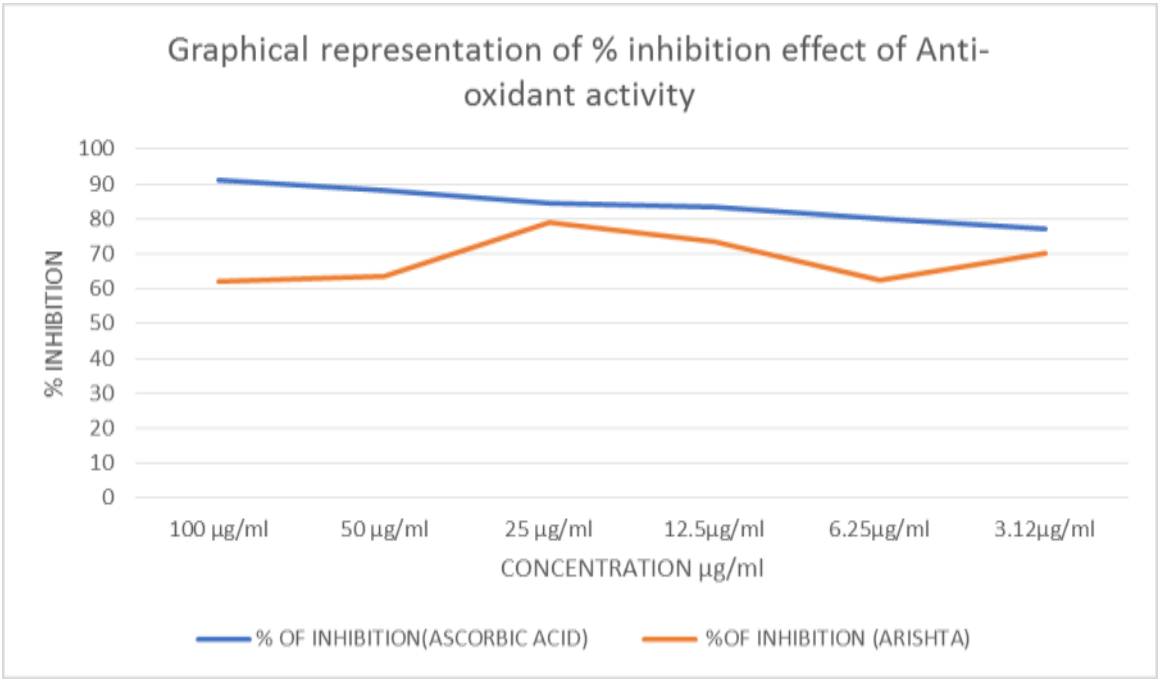


Figure 6: Graphical representation of Anti-oxidant activity.

Anti-inflammatory activity

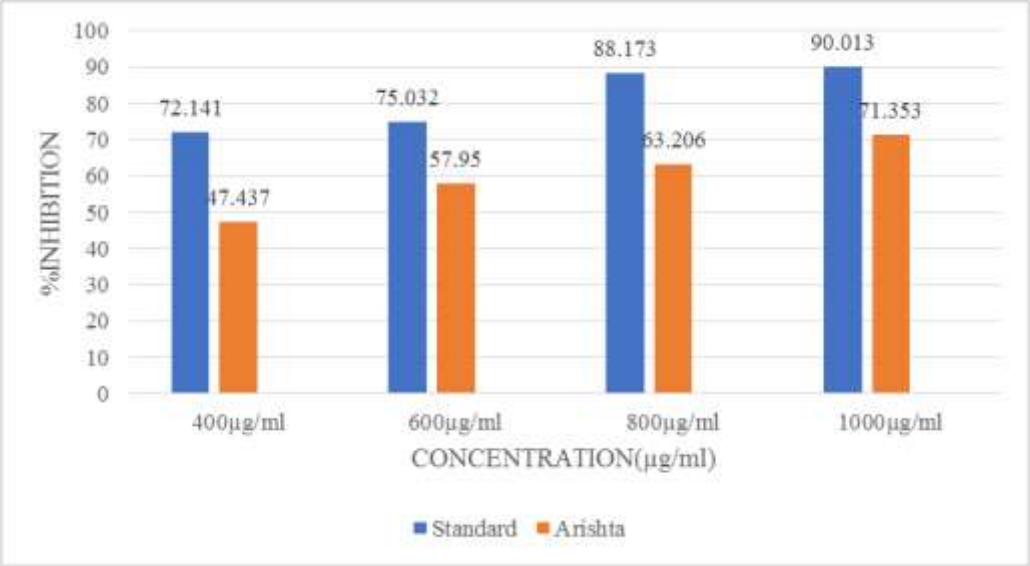


Figure 7: Graphical representation of the anti-inflammatory activity.

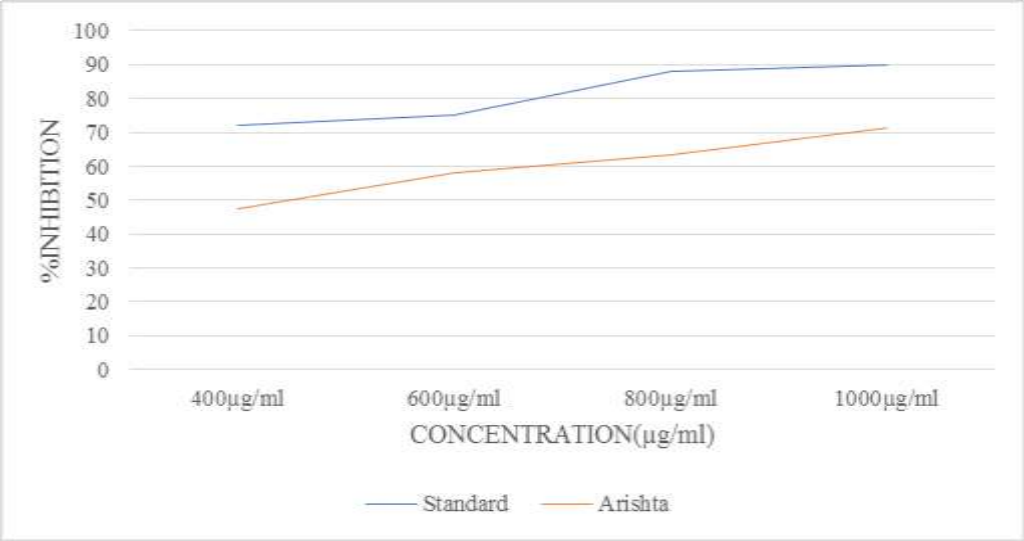


Figure 8: Graphical representation of anti-inflammatory activity.

CONCLUSION

In India Ayurvedic drug industry is rapidly growing and many herbal products are released in to the market. The implementation of appropriate standardization is necessary to guarantee the safety and efficacy of these formulations. Arishta is a traditional medicine which is currently available in market. The physicochemical evaluation helps to determine the physical and chemical properties of these formulations. Biological evaluation involves studying the impact of formulation on living organisms. By doing the physicochemical and biological evaluation of arishta for ensuring the quality, purity, safety, and efficacy of this

formulations. Evaluation include chemical, microbial, and organoleptic aspects, ensuring quality and adherence to ayurvedic principles.

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