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RESEARCH ARTICLE

Evaluation of Anti-Bacterial Activity of Combined Natural Products

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ABSTRACT

Background: *Aloe vera*, turmeric, and *hibiscus* are plants with high medicinal properties. In this study, we aimed to investigate the antibacterial activity by performing preliminary tests, including thin layer chromatography (TLC) and zone of inhibition. **Materials and Methods:** *A. vera* leaves, turmeric rhizomes, and *hibiscus* leaves are dried and subjected to extraction by maceration using methanol as a solvent. The percentage yield of each extract was determined. Phytochemical screening was performed to identify various bioactive compounds, including carbohydrates, flavonoids, tannins, phenolics, and fats. The extracts were further evaluated for TLC for separation and identification of active components. Then the extracts were subjected to zones of inhibition to determine the highest antibacterial activity of the plant. **Results:** Methanolic extract exhibited the highest percentage yield. Phytochemical screening revealed the presence of carbohydrates, flavonoids, tannins, and phenolics. TLC indicates the presence of active compounds in the extracts, and the zone of inhibition is known by measuring the area around a sample where bacterial growth is inhibited; the larger the zone of inhibition, the stronger the antibacterial activity. **Conclusion:** The antibacterial activity of combined natural plant products was evaluated, and it was found that compared to individual plant products, combined plant products show higher antibacterial activity.

Keywords: *Aloe vera*, *Hibiscus* and antibacterial activity, Turmeric

INTRODUCTION

From a plant's tissues, cells, and secretions, natural plant products are harvested. Global suppliers of botanical ingredients and technical services to the personal care sector include natural plant products. The natural products that plants make are abundant and diverse, and these substances serve crucial ecological purposes by protecting plants from numerous environmental stresses such as bacteria, fungi, pests, illnesses, UVB radiation, and other pests and diseases. Various ailments such as diabetes, rheumatism, cancer, ulcers, cough, and inflammation are treated with natural plant compounds.[1]

MATERIALS AND METHODS

Aloe vera

50 g of *A. vera* leaves are cut into small pieces and kept under sunlight for drying for 5 days. Then, collect the dried pieces and grind them to get a fine powder.

About 10 g of *A. vera* powder was weighed and dissolved in 50 mL of methanol in a conical flask [Figure 1], covered with silver foil. The preparation was kept undisturbed for 7 days, with stirring at regular intervals. After 7 days filtered using

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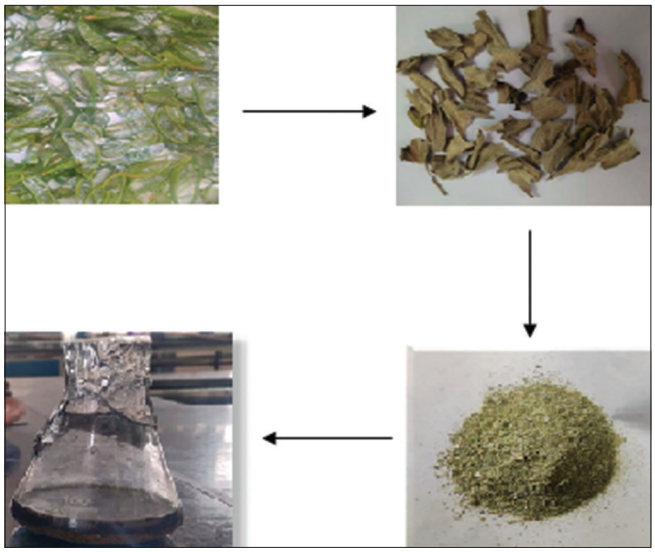

Figure 1: Extraction of *Aloe vera*

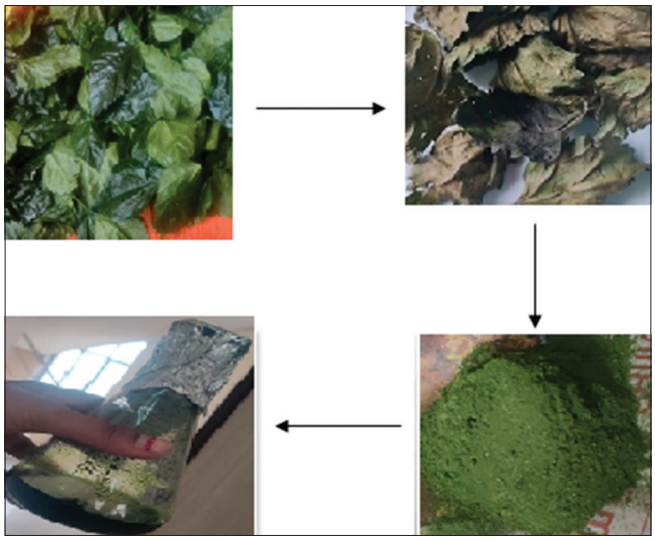

Figure 2: Extraction of *Hibiscus*

Whatman filter paper, the filtrate was collected and stored under humid conditions.[2-6]

Hibiscus

20 g of *hibiscus* leaves are kept under sunlight for drying for 4 days. After 4 days, collect the dried leaves and grind them into a fine powder.

About 10 g of dried *hibiscus* powder was dissolved in 50 mL of methanol in a conical flask [Figure 2] and wrapped in silver foil. It was kept aside for 7 days, stirring at regular intervals. After 7 days, it was filtered using Whatman filter paper. The filtrate was collected and stored under humid conditions.[7-11]

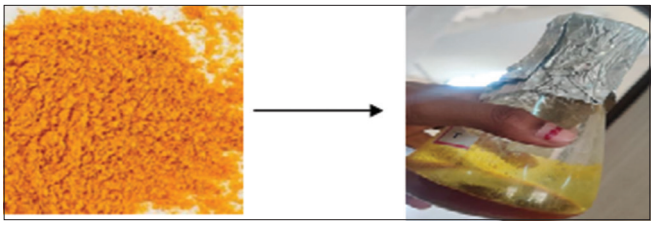

Figure 3: Extraction of turmeric

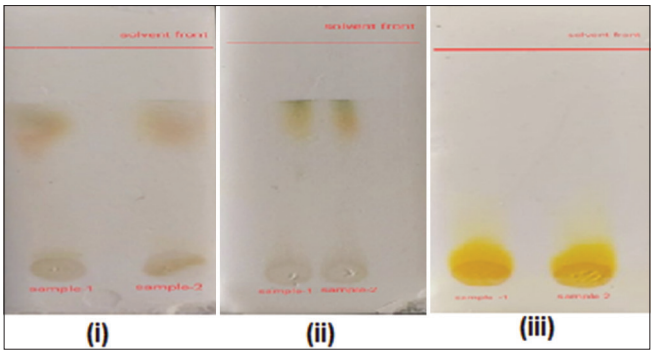

Figure 4: Thin layer chromatography of (i) *Hibiscus*, (ii) *Aloe vera* and (iii) Turmeric

Turmeric

20 g of turmeric rhizomes are taken and grinded to get a fine powder.

About 10 g of turmeric powder was dissolved in 50 mL of methanol in a conical flask [Figure 3] and covered with silver foil. This preparation is kept undisturbed for 7 days, with stirring at regular intervals. After 7 days, it was filtered using Whatman filter paper. The filtrate was collected and stored under humid conditions.[12-16]

RESULTS AND DISCUSSION

Thin layer chromatography (TLC)

TLC was done using silica gel as the stationary phase and hexane (4), ethyl acetate (5), and methanol (1) as the solvent system. The Rf values were found to be 0.08 (*Hibiscus*), 0.6 (*A. vera*), and 0.7 (Turmeric) as shown in Figure 4.

To determine the presence of curcuminoids in turmeric, alloin and barbaloin in *A. vera*, and anthocyanins in *Hibiscus*, the extracts are submitted to phytochemical screening.[5] The results are compiled in Table 1.

Zone of inhibition

Preparation of nutrient agar

Nutrient agar was prepared by adding 7 g of nutrient agar to 250 mL of distilled water. The mixture was stirred to dissolve the components and boiled for 30 min. The prepared nutrient agar was poured into each petri plate, and the plates were left on a sterile surface until the agar solidified. The prepared nutrient agar plates were incubated at 37°C for 24 h. The nutrient agar was inoculated with gram-positive *Streptococcus* and gram negative bacteria of *Escherichia coli* by pipetting out 10 µL of respective culture on plates and spreading over the nutrient agar plates using a sterilized spreader.[2]

Procedure

Sterile agar (at 45°C) was poured into a sterile Petri dish that had been inoculated with the test organism. Plates allowed to dry for an hour. Wells (10 mm) were made with the help of a framed cork borer on the surface of the agar plates. About 0.1 mL of extract is delivered in each well. And incubated at 37°C for 24 h. The zone of inhibition was measured and calculated against the zone of inhibition of azithromycin used as a standard. The Zones of inhibition of combined compounds and single compounds are shown in Figures 5-10, and

Table 1: Phytochemical analysis

Test	*Hibiscus*	Turmeric	*Aloe vera*
Molisch	+	+	+
Benedict's	+	+	+
Dragondroff's	+	+	+
Wager's	+	+	+
Legal's	+	+	+
Shinoda	-	+	+
Salkowski	+	-	-
Foam test	+	+	+
Ninhydrin	+	+	+
Biuret test	+	+	+

+: Present, -: Absent

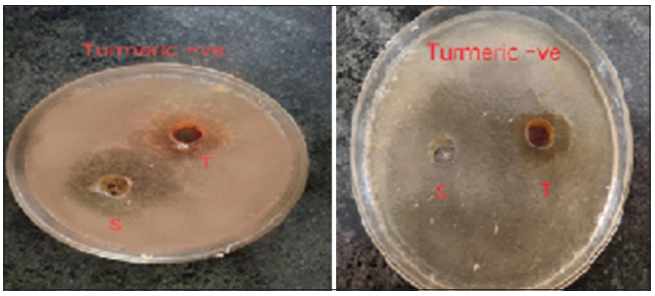

Figure 5: Zone of inhibition of turmeric (gram +ve and gram -ve)

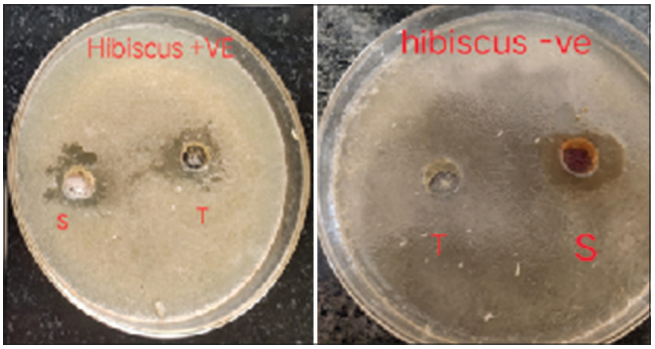

Figure 6: Zone of inhibition of *Hibiscus* (gram +ve and gram -ve)

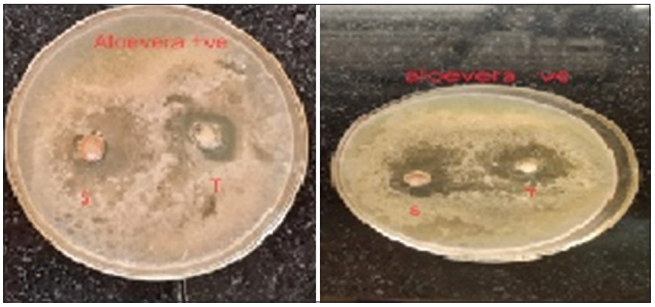

Figure 7: Zone of inhibition of *Aloe vera* (gram +ve and gram -ve)

Table 2: Zone of inhibition

Name of compound	Concentration (µg/mL)	Zone of inhibition (mm)	
		Streptococcus	*Escherichia coli*
Azithromycin (Standard)	10	15	15
Hibiscus		6	7
Turmeric		9	8
Aloe vera		7	7
***Al**oe vera-Hibiscus*		12	10
Aloe vera-turmeric		13	11
Hibiscus-turmeric		13.5	12.5

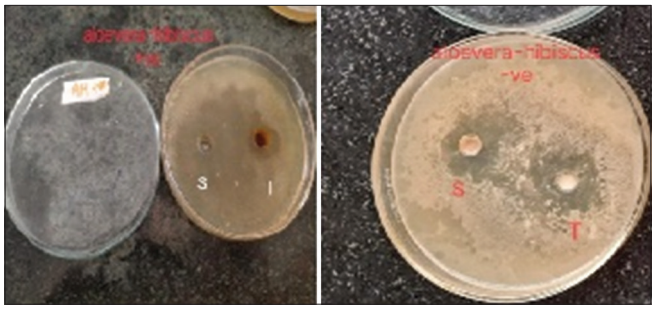

Figure 8: Zone of inhibition of *Aloe vera -Hibiscus* (gram +ve and gram -ve)

Figure 9: Zone of inhibition of *Aloe vera* - turmeric (gram +ve and gram -ve)

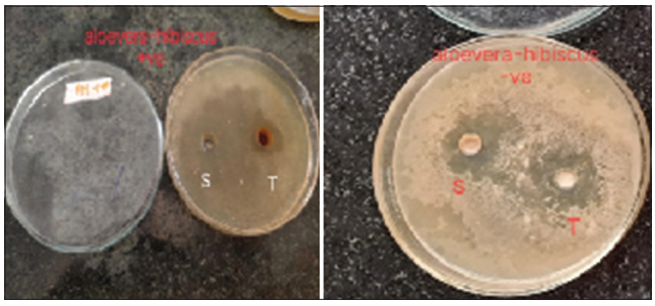

Figure 10: Zone of inhibition of *Hibiscus*- turmeric (gram +ve and gram -ve)

Table 2 provides information about compounds, concentration, and measured zones of inhibition of Azithromycin, *Streptococcus,* and *E. coli*.[4]

Combinations

Anti-microbial activity of zone inhibition of combined extract of Aloevera-Hibiscus (gram +ve and gram -ve)Aloevera-turmeric (gram +ve and gram -ve), Hibiscus-turmeric (gram +ve and gram -ve) can be seen from Figure 8-10.

CONCLUSION

The results revealed significant and varying zones of inhibition for the different extracts, indicating their effective bacterial growth. Taking preliminary tests, TLC and zone of inhibition strongly support the antibacterial activity of these extracts. The antibacterial activity of turmeric, *A. Vera,* and *hibiscus* has been validated through multiple experimental approaches. When compared to individual compounds, a combined extract has shown more zone of inhibition from *hibiscus-turmeric* 13.5 mm–12.5 mm. These natural extracts hold promise as effective and safe antibacterial agents, opening new avenues for the development of alternative treatments against bacterial infections.

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