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Formulation & evaluation of herbal spray containing *Coleus amboinicus* Lour & *Melia azedarach* Linn for antibacterial and antifungal activities

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Abstract

Developing countries like India, rely on medicinal plants for various ailments. India is known for rich heritage of medicinal plants and its usage of herbals to treat disease conditions mentioned in a traditional literature and the system like Ayurveda, Siddha, Unani practiced by our ancestors. Now, there is a great demand for the herbs due to the emergence of numerable diseases based on lifestyle and many factors. Medicinal plants can be utilized by a common man to the rich people, in the form of herbs (home), kitchen herbs and as Nutraceuticals. Based on the therapeutic values of herbs, we attempted for the herbal face mask which will provide significant protection against organisms like *Streptococcus aureus*, *E. coli* & *Candida albicans*, *Aspergillus niger*. In this work, we have taken leaves of medicinal plants *Coleus amboinicus* & *Melia azedarach*.

Keywords: Medicinal plants, *Coleus Amboinicus*, *Melia Azedarach*, Herbal face mask, Antibacterial, Antifungal

Introduction

Developing countries like India depends on herbal medicines for their healthcare as per WHO. India having rich heritage of herbal resources and the traditional systems of medicines still in practice for various healthcare need by the people. Because of the least side effects and cost effectiveness, herbal medicines are in high demand and their acceptance level has been increasing day by day. In Worldwide, there are 50,000 to 80,000 flowering plants are used medicinally^[1].

COVID-19 / Need of Face masks

From the beginning of the century, we had gone through many pandemic outbreaks on many respiratory infections like influenza, Ebola etc. In this current situation Coronavirus disease (COVID-19) is an infectious disease caused by the SARS-CoV-2 virus. The World Health Organization (WHO) advises using masks for prevention and control measures to prevent the spread of COVID-19. Even though the mask is weared properly it should not provide adequate protection for the people. Masks can be used either for the protection of healthy people or to prevent onward transmission.

Herbal face mask

Normally a mask acts as a mechanical barrier which helps to protect from large particles to small particles, coughing, sneeze, splash or spraying of fine droplets. Using herbs in mask helps to provide some additional activities like antibacterial, antiviral, antifungal, bronchodilation, improves respiratory capacity etc. Some herbs may also act as an immune stimulant which helps to increase the immunity of our body naturally. Using herbal drugs instead of chemical in masks may cause some skin irritations, adverse drug reaction, allergic reactions etc. So, using herbs instead of chemicals will leads to reduce this allergic reaction. There are five basic classifications of masks present. They are listed below surgical mask, activated carbon mask, respirators, reusable cloth mask, herbal mask.



Fig 1: Surgical masks



Fig 2: Activated carbon masks



Fig 3: Types of Respirators



Fig 4: Reusable cloth masks



Fig 5: Herbal face masks

In this present work, the herbs *Coleus amboinicus* and *Melia azedarach* and Thyme, Clove and Lavender oils together mixed in the proper ratio, herbal spray prepared and evaluated for antibacterial and antifungal properties attempted using Face mask for its improved performance and presented here [14].

Plant Description



Fig 6: *Coleus amboinicus* Lour



Fig 7: *Melia azedarach* Linn



Fig 8: *Syzygium aromaticum*



Fig 9: *Lavandula stoechas*



Fig 10: *Thymus vulgaris*

Materials and Methods - Plants selected for the study

Based on a literature survey, we have selected *Coleus amboinicus*, *Melia azedarach* for the preparation of herbal spray for applying in the herbal face mask. Coarsely powdered herbal material like leaves & root bark placed inside a container, the menstruum is poured on top until completely covered the drug material and allowed for maceration process for three days.

Preparation of herbal spray

Preparation of herbal spray having the following composition After taking the extract from the herbal plant we have dissolved 2 gm of each extract in 10ml distilled water. On completing this process then we added different types of oil to it. The list of oils is mentioned below

1. Clove oil.
2. Lavender oil.
3. Thyme oil.

3 drops of clove oil, 4-5 drops of lavender oil, and 3 drops of thyme oil were added in each extract. Then the mixture was shaken well for proper mixing and placed in a sprayed bottle.

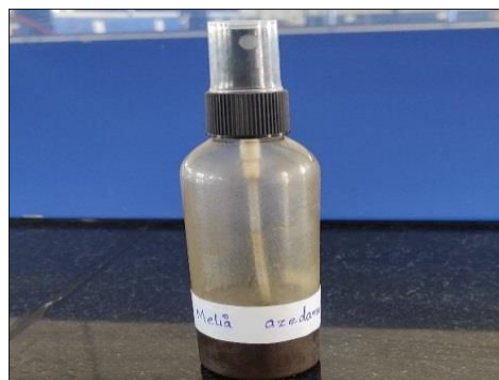
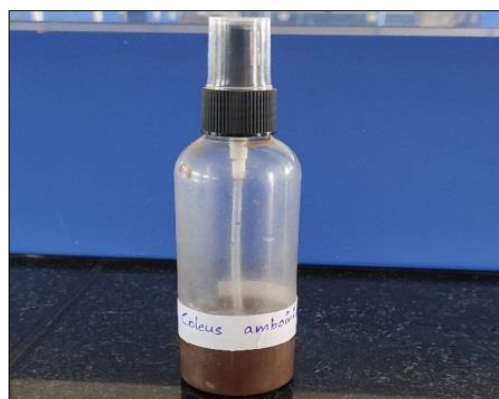


Fig 11: Spray bottle filled with *Coleus amboinicus* extract & *Melia azedarach* extract

Preparation of mask: Procurement and Stitching of Cloth Mask



Fig 12: Measurements

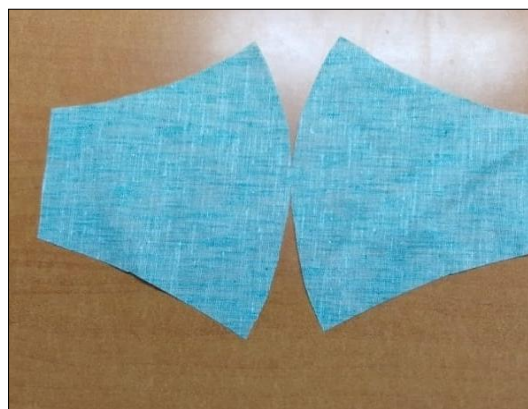


Fig 13: Cloth cut as per the shape



Fig 14: Stitched masks



Fig 15: Outer side of the mask

Applying herbal spray on mask

After the preparation of herbal spray, we impregnated the herbal spray on the cotton mask and dried it for 10-15 minutes.

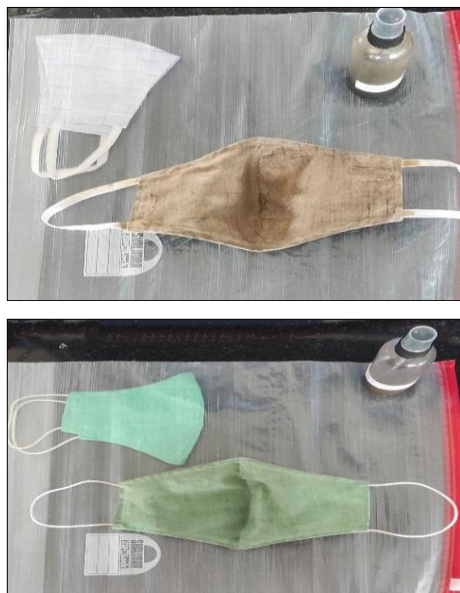


Fig 16: Face mask with *Melia azedarach* extract & *Coleus amboinicus* extract

Evaluation of anti-microbial activity of herbal spray
1. Agar-well diffusion method Anti-bacterial activity of *Melia azedarach* Linn & *Coleus amboinicus* Lour
Procedure

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hours culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (*E. coli* and *S. aureus*) Wells were cut and concentration of samples

MZ (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37 °C for 24 hours.

The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

Antifungal activity of *Coleus amboinicus* – Agar - Well Diffusion Method

The potato dextrose agar medium was prepared by dissolving 40 gm of potato infusion, 4 gm of dextrose and 3.5 gm of agar in 200 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121 °C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten.

Procedure

Petri plates containing 20 ml potato dextrose agar medium was seeded with 72hr culture of fungal strain (*A. niger* and *C. albicans*) wells were cut and different concentration of sample CAP (500, 250, 100 and 50 µg/ml) were added. The plates were then incubated at 37 °C for 48-72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B (100 units) was used as a positive control. The values were calculated using Graph Pad Prism 6.0 software (USA).

Results and Discussion

1) Anti-bacterial activity a) Anti-bacterial activity of *Coleus amboinicus*

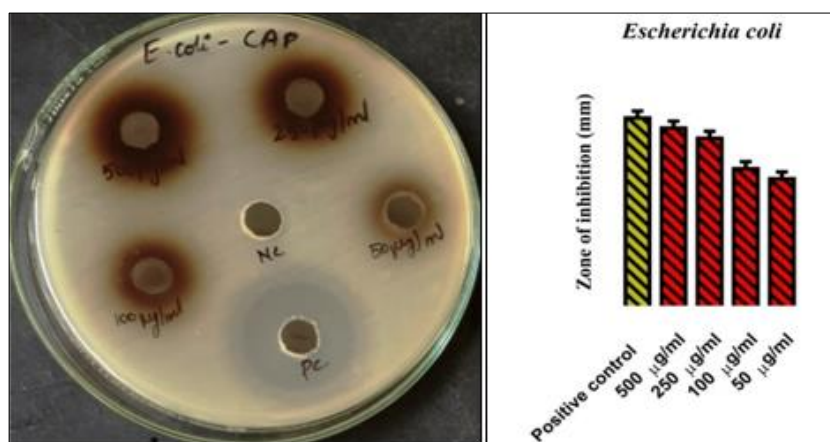


Fig 17: Effect of sample *Coleus amboinicus* against *E. coli*.

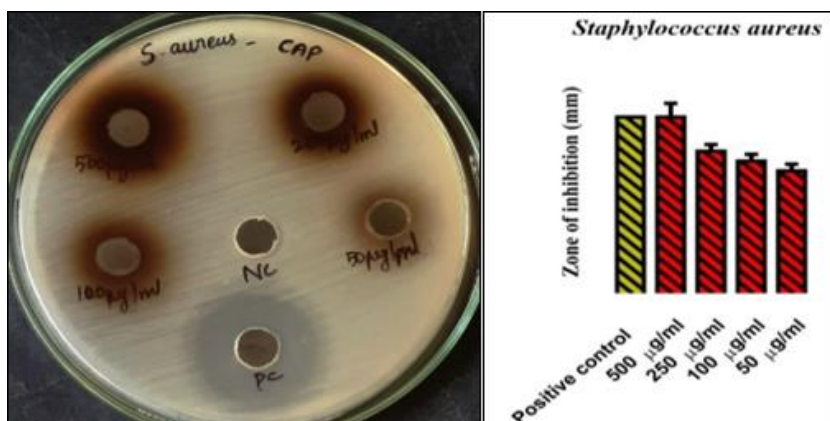


Fig 18: Effect of sample *Coleus amboinicus* against *S. aureus*.

Table 1: SD± Means of zone of inhibition obtained by samples *Coleus amboinicus* against *E. coli* and *S. aureus*.

S. No.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				PC
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	
1.	<i>E. coli</i>	<i>Coleus amboinicus</i>	17.5±0.7	16.5±0.7	13.5±0.7	12.5±0.7	18±1.4
2	<i>S. aureus</i>	18±1.4	18±1.4	14.5±0.7	12.5±0.7	11.5±0.7	18±0

b) Anti-bacterial activity of *Melia azedarach*

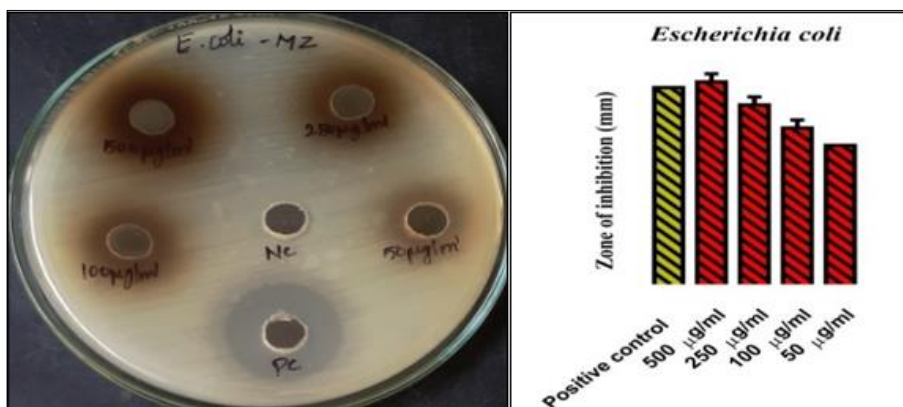


Fig 19: Effect of sample *Melia azedarach* against *E. coli*.

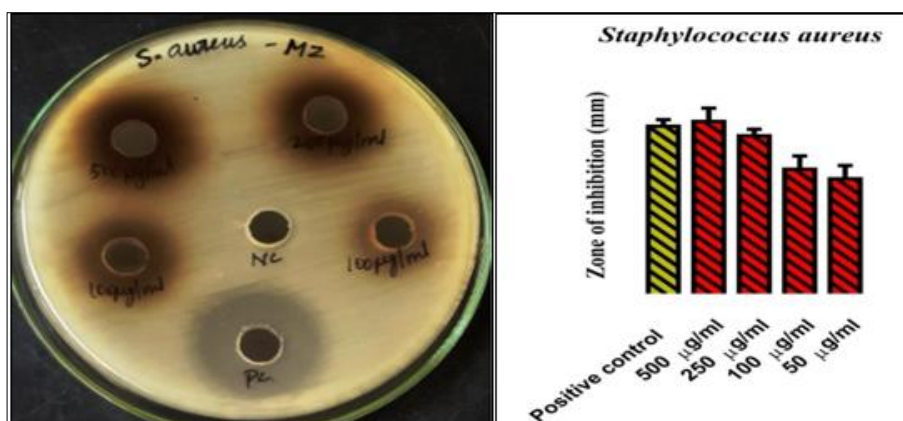


Fig 20: Effect of sample *Melia azedarach* against *S. aureus*

Table 2: SD± Means of zone of inhibition obtained by samples *Melia azedarach* against *E. coli* and *S. aureus*.

S. No.	Name of the test organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				PC
			500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	
1.	<i>E. coli</i>	<i>Melia azedarach</i>	17.5±0.7	15.5±0.7	13.5±0.7	13.5±0.7	17±0
2	<i>S. aureus</i>	18±1.4	18±1.4	16.5±0.7	13±1.4	12±1.4	17.5±0.7

2) Antifungal activity - a) Antifungal activity of *Coleus amboinicus*

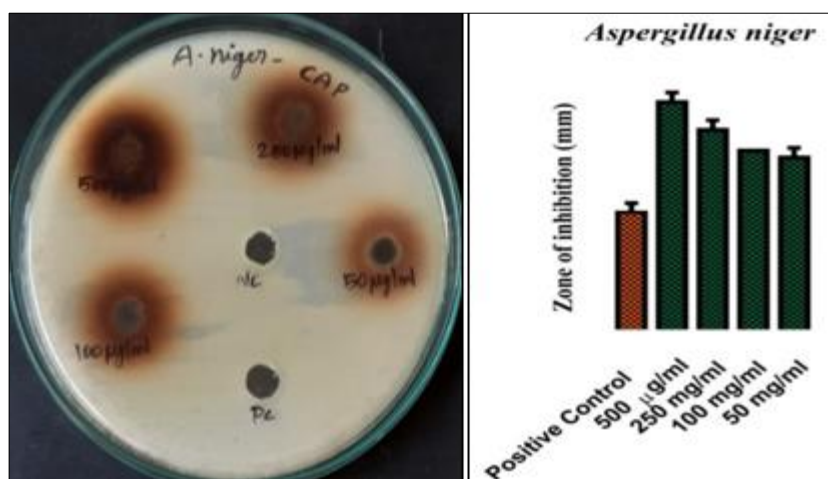


Fig 21: Effect of sample *Coleus amboinicus* against *A. niger*.

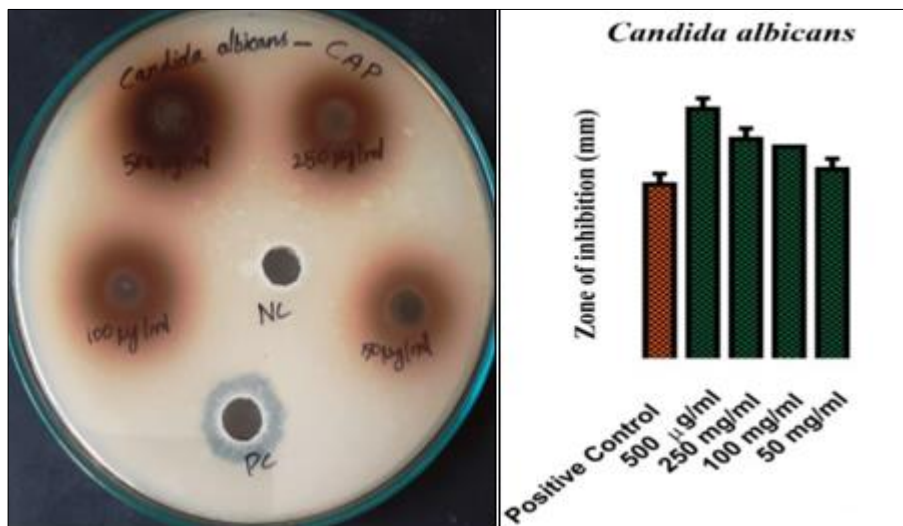


Fig 22: Effect of sample *Coleus amboinicus* against *C. albicans*.

Table 3: SD± Means of zone of inhibition obtained by sample *Coleus amboinicus* against (*A. niger* and *C. albicans*).

S. No	Name of the organism	Name of the test sample	Zone of inhibition (Mm) SD ± MEAN				
			PC	500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml
1	<i>A. niger</i>	<i>Coleus amboinicus</i>	8.5±0.7	16.5±0.7	14.5±0.7	13±0	12.5±0.7
2	<i>C. albicans</i>		11.5±0.7	16.5±0.7	14.5±0.7	14±0	12.5±0.7

b) Antifungal activity of *Melia azedarach*

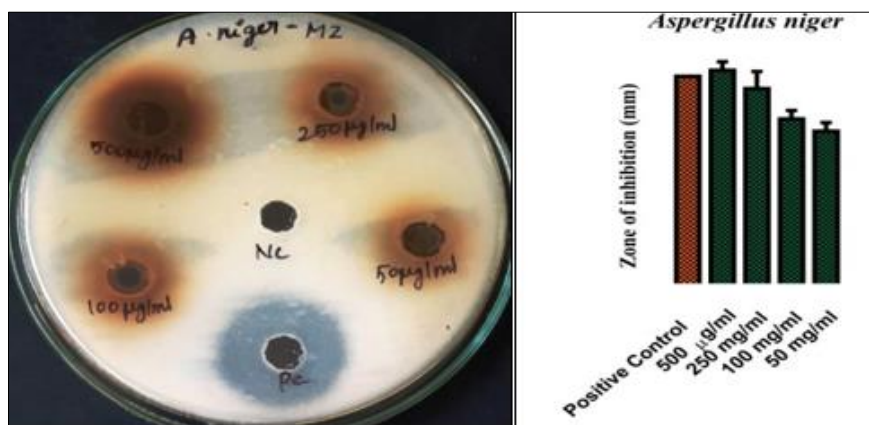


Fig 23: Effect of sample *Melia azedarach* against *A. niger*.

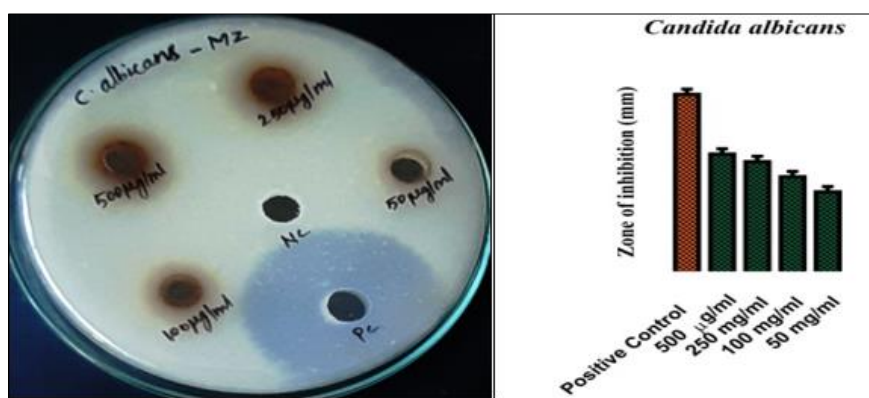


Fig 24: Effect of sample *Melia azedarach* against *C. albicans*.

Table 4: SD± Means of zone of inhibition obtained by sample *Melia azedarach* against (*A.niger* and *C. albicans*)

S. No.	Name of the organism	Name of the test sample	Zone of inhibition (mm) SD ± MEAN				
			PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1	<i>A. niger</i>	<i>Melia azedarach</i>	17±0	17.5±0.7	16±1.4	13.5±0.7	12.5±0.7
2	<i>C. albicans</i>		23.5±0.7	15.5±0.7	14.5±0.7	12.5±0.7	10.5±0.7

Discussion

The extracts of *Melia azedarach* and *Coleus amboinicus* tested for Antibacterial and antifungal activities. Here the aqueous extract of *Melia azedarach* showed significant inhibition with 500 µg/ml against the bacterial strains *E. coli* & *Staphylococcus aureus* compared with the standard Gentamicin. Results presented in Table no: 3 & Zone of inhibition presented in Figure no: 19, 20.

The aqueous extract of *Coleus amboinicus* was tested for antibacterial and antifungal activities. Results presented in Table no: 2 The extract at a dose of 500 µg/ml effective against the bacterial strains *E. coli* & *Staphylococcus aureus*. Compared with the standard Gentamicin. Zone of inhibition 500µg/ml presented in Fig no: 17, 18.

Both the Extracts of *Melia azedarach* and *Coleus amboinicus* effective an against the fungal strains *Candida albicans* & *Aspergillus niger* compared with the standard Amphotericin. Results are presented in Table no: 4, 5 & Zone of inhibition 500 µg/ml presented in Figure no: 21, 22, 23, 24.

The extracts contain Alkaloids, Tannins, Glycosides and Saponins. The activity might be due to the synergistic action of the phytoconstituents present in the extracts. Based on literature, both the extracts were found useful in treating viral, bacterial and fungal infections like COVID -19.

Conclusion

Based on the survey and the studies revealed that the aqueous extracts of *Coleus amboinicus* & *Melia azedarach* showed significant antibacterial and antifungal activities. As these herbal extracts found useful in treating the infections, can effectively use as a spray which will give additional support to the people who wear this type of herbal masks in fighting against several disease-causing microbes and viral infections like COVID-19 & other respiratory illness.

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