"Antibacterial activity of Herbal Gau Dhoopbatti as an Air Purifier and Disinfectant Material for Utensils"

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Article Info:

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Acknowledge to the Idea Originator: The idea Originator Jv'n Dr. Panckaj Garg always strives for equal distribution of community needs and through this project findings he provides information related to Herbal Gau Dhoopbatti antibacterial activity for air cleansing and Disinfectant surface. This project also suggests the informative details of Antibacterial activity of Herbal Gau Dhoopbatti and its uses in for aircleansing and disinfectant surface through inhibiting Pathogenic bacteria.

ABSTRACT

In a variety of religious practices such as havans, cow dung has been used in command to cleanse the environment. With an plan to reduce the practice of chemicals or disinfectants to cleanse the environment, efforts were completed to work out an Herbal Gau Dhoopbatti using cow dung, cow urine, and a range of other medicinal plant powders with medicinal and antibacterial properties such as Neem, Arjuna, Jamun, Bhadraksh, Champa and Hajara. The current study focused on preparation and assessment of natural and Herbal Gau Dhoopbatti formulation for air cleansing. In this study, the antibacterial activity of the two formulations of dhoopbatti was analyzed and it was established that it inhibits the growth of air borne bacteria. So, it can be a budding source for disinfection of air. Ash of Dhoopbatti is also useful due to cow dung components. It can be used as disinfectant to clean daily basis utensils due to its Antibacterial activity. Two Ingredients Neem and Arjuna have reported Antiviral activity also. So this study also suggests that as it is Antibacterial as well as it can help in controlling Viral infection also.

Keywords- Antibacterial activity, cow dung, Cow Urine, Neem, Arjuna, Jamun, Bhadraksh, Champa and Hajara

INTRODUCTION-

The environment has been a main alarm in current scenario. The steady pollution has gathered the thought of numerous populaces. Fresh surroundings include clean air, water, soil and energy, is necessary for human survival (Clean Air Act2007 a, b). Microbes of the air generate various airborne diseases. Disease-causing pathogens are organisms that multiply from an infected person to another during coughing, talking and sneezing. These disease causing bacterias can be *Staphylococcus*, *Streptococcus*, *Mycoplasma pneumonia*, *Chlamydia pneumonia*, *Bordetella pertussis*, *Pseudomonas aeruginosa*, *Pseudomonas mallei*, *Mycobacterium tuberculosis*, *Corynebacteria diphtheria* etc. Various efforst has been taken up for cleansing air to make free or less airborn pathogens such as chemical treatment but there are side effects of their applications (Cleaning and the Environment 2012).

Consequently, the current study focuses on the progress of a Herbal Gaau Dhoopbatti and its Antibacterial activity, which can be successfully used for inhibits the Bacterial growth. Two mixture formulations were developed using natural componants such as cow dung, cow urine and various parts of Medicinal plants which are known traditionally for medicinal and antibacterial activity.

MATERIAL AND METHODS REQUIRED

- All the plant powders were Prepared (Neem, Zamun, Arjuna, Bhadraksha, Champa and Hajara) from dry leaves and flower part collected from Harbel garden of Jayodi Viidyapeeth Women's University, Jaipur.
- Cow dung and urine was collected from Pashu Ashram of University Campus. Two types of Dhoopbatti were prepared.
- Mixture I is having Cow Dung, Cow Urine, Neem, Arujna, Jamun and Bhadraksh (Table-1).
- Mixture II is having Cow Dung, Cow Urine, Neem, Champa, Hajara and Bhadraksh (Table 2).

Sr. No	Ingredient	Scientific Names	Plant part used	Quantity
1	Cow dung	-	-	850 gram
2	Cow Urine	-	-	500 ml
3	Neem	*Azadirachta indica*	Leaves	100 gram
4	Arujna	*Terminalia arjuna*	Leaves	100 gram
5	Jamun	*Syzygium cumini*	Leaves	100 gram

6	Bhadraksha	*Guazuma ulmifolia* Lam.	Leaves	100 gram

Table – 1 Details of Ingredients of Mixture I Gau Dhoopbatti (Collected From Herbal Garden of Jayoti Viidyapeeth Women's University, Jaipur)

Sr. No	Ingredient	Scientific Names	Plant part used	Quantity
1	Cow dung	-	-	850 gram
2	Cow Urine	-	-	500 ml
3	Neem	*Azadirachta indica*	Leaves	100 gram
4	Champa	*Michelia champaca*	Flower and Leaves	100 gram
5	Hajara	*Tagetes erecta*	Flower and Leaves	100 gram
6	Jamun	*Syzygium cumini*	Leaves	100 gram
7	Bhadraksha	*Guazuma ulmifolia* Lam.	Leaves	100 gram

Table – 2 Details of Ingredients of Mixture II Herbal Gau Dhoopbatti (Collected From Herbal Garden and Pashu Ashram of Jayoti Vidyapeeth Women's University, Jaipur)

Preparation of Dhoopbatti at JVWU, Jaipur Campus

- Fresh cow dung collected from Pashu Ashram of University, was taken in a clean area. Respective plant powders with their respective quantity were added to Cow dung
- Cow urine collected from Pashu Ashram of University, was added into it and again macerated finely to obtain a fine paste. Dhoop sticks were prepared manually.
- These dhoopbatti were dried for a day and then stored in an air sealed pouches with labelling (Figure-1).

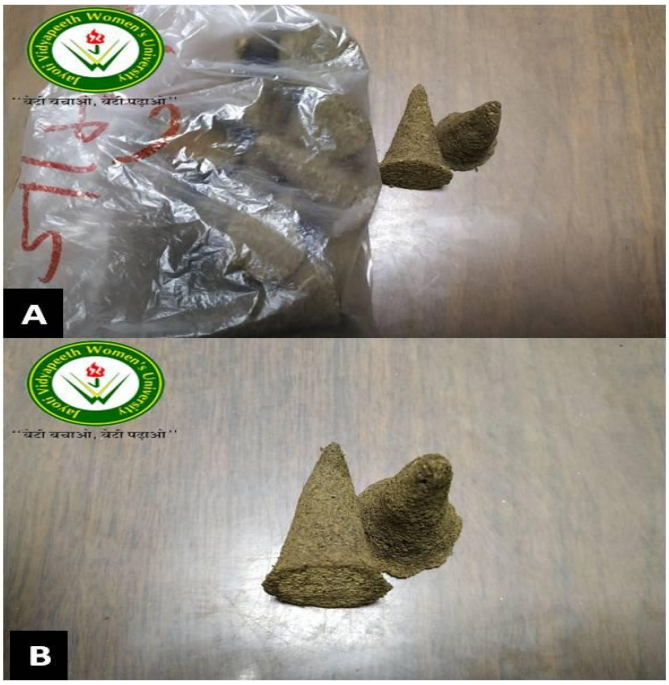

Figure- 1 (A) Herbal Gau Dhoopbatti- I (Mixture- I) (B) Herbal Gau Dhoopbatti- II (Mixture- II)

Phytochemical composition of All ingredients-

1. **Cow dung-**
    - Cow dung has Organic material 14.6 (%), Total nitrogen 0.30–0.45 (%), Total phosphorus 0.15–0.25 (%), Total potassium 0.05–0.15 (%), Flavanoids, Glycosides, , steroids, Tannins, Phenols which has antibacterial activity (Rajeswari et al, 2016).
    - As cow urine reported remarkable antibacterial activity against the pathogenic bacteria, for which it can be selected for further studies to isolate bioactive natural constituents that may address to unmet therapeutic need (Shah et al, 2011).
    - The medicinal properties of cow dung has been reported to prepare drugs for quite a lot of diseases caused by antibiotic resistant pathogenic microorganisms (Reddy et al, 2013; Rajeswari et al, 2016).
    - The extract of cow dung ash reported highly basic with pH of 11.7 and the elements fluctuate in the following decreasing order of concentration; K>Na>Mg>Ca>Fe>Al>Zn. This extract reported effective antibacterial activity (Waziri and Suleiman, 2013).

- The cow, according to the Vedas, gives three products for human benefits: (i) Godugdha (cow milk) (ii) Goghruta (ghee) (iii) Gomutra (urine). Eight types of urine are used for medicinal purpose, among which cow urine is held to be the best (Pachori et al, 2013).
- One report have reported about dry cow dung powder consisting humic acid. Which is also a antiviral, antimicrobial agent (Barot N.S. and Bagla H.K. 2009)

2. Cow Urine-

It has Water – 95% Urea – 2.5% Minerals, Salts, Hormones, Enzymes – 2.5% Healthy cow urine has volume of 17-45 ml/Kg/day with specific gravity ranging from 1.025-1.045. Its pH ranges between 7.4 to 8.4 with seasonal variations. Urea nitrogen and Total nitrogen varies between 23-28 ml/kg/day and 40-45 ml/kg/day respectively.

In healthy cows' urine does not contain protein, glucose and haemoglobin. **Urea is a Strong Antimicrobial Agent and it is end protein metabolism, while uric acid has antimicrobial activity and it helps to control infections.** Other important ingredients with their functions are as follows

- **Creatinine - it acts as an Antibacterial- Volatile compound (**Devenport et al 2014).
- **Aurum hydroxide - Antibacterial, improves immunity, acts as antidote**
- Enzymeurokinase - It is accountable for dissolving the blood clot, curing of heart disease, blood circulation
- Colony Stimulating factor - effectual for cell division & multiplication
- Erythropoietin stimulating factor is major stimulating factor for production of Red blood cells.
- Gonadotropin - Promotes menstrual cycle, sperm production
- Anticancer substances- Prevents multiplication of carcinogenic cells. (Harshad et al 2017)
- Cow's urine is extensively used in the Ayurvedic pharmaceuticals for elevating the properties of many drugs, by giving bhavana (repeated trituration). In shodhana (purification) of metals used in therapeutics, cow urine was lengthily used. Charka, Sushruta and all other ancient physicians have given major importance to cow's urine (Ahuja etal, 2012).

3. **Neem (*Azadirachta indica*)-**

- Volatile compounds were identified in three distinct chemical classes (Monoterpenoid, sesquiterpenoid and purine nucleosides).
- **γ-Elemene (24.06%), 3,7 (11)-eudesmadiene (6.83%), caryophyllene (6.40%), and 10s,11s-himachala-3(12),4-diene (6.36%)** were the major components of neem leaf volatiles, followed by other compounds present in less than 4% which might be accountable for diverse biological activities experiential such as Antimicrobial activity. Secondary metabolite is Azadirachtin present in Neem (Perera et al, 2019).
- Neem Bark has been investigated for its Antiviral activity against Herpes simplex virus type-1 (HSV-1) (Tiwari et al, 2010).

4. **Arjuna *(Terminalia arjuna)*-**

It has tannins, terpenoids, **alkaloids- Volatile Compounds,** glycosides, flvanoids and **phenols-Volatile Compounds** Which have been found to have antimicrobial properties (Aneja et al, 2012).

Volatile compounds have also been identified in arjuna such as Major constituents of leaves :

- **carvacrol (11.17%),**
- **thymol (6.52%),**
- **α-terpinyl acetate (5.92%) and anethole (5.13%)**

Other than these compounds more other volatile compounds are presents- Decamethylcyclopentasiloxane, Benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester, Anethole, Dodecamethylcyclohexasiloxane, Globulol, Cadinol etc (Chatha et al, 2014; Amalraj and Gopi, 2016; Gupta and Kumar 2017).

- Carvacrol, thymol, α-terpinyl acetate and anethole has strong antibacterial activity properties (Bano et al, 2019).
- Casuarinin, a hydrolyzable tannin isolated from the bark of *Terminalia arjuna* Linn. (Combretaceae), was reported for its antiviral activity on herpes simplex type 2 (HSV-2) *in vitro* (Cheng et al., 2002).

5. **Bhadraksha (*Guazuma ulmifolia* Lam.):**

The Bhadraksha (*G.ulmifolia)* has been revealed to have constituents such as octacosanol, taraxeroloac, friedelin-3- áoac, â-sitosterol, and Friedelinol-3-acetate, tartaric acid or tannins such as catechins, colatannins and caffeine in leaves,

kaempferol in heartwood while bark contains friedelin, betulin, â-sitosterol and it is a wealthy basis of tannins (Kumar and Gurunani, 2019). The oil of *Guazuma ulmifolia* Lam. Leaves was studied by GC and GUMS. The chief components established were precocene I (56.0%), P-caryophyllene (13.7%) and (2Z,bE)-farnesol (6.6%) and precocene I and P- caryophyllene has reported antimicrobial activity (Adaga et al, 1997).

6. **Jamun (*Syzygium cumini*):**

- It is indigenous to India has been used as antimicrobial activity for century in Unani and Ayurveda.

  The presence of secondary metabolic compounds such as **Alkaloids- Volatile Compounds** tannins, **phenols- Volatile Compounds- Antibacterial agents,** lipids, flavonoids
- **Volatile Oils** - Volatile oils are very multifaceted mixtures of compounds. The ingredients of the oils were mainly **monoterpenes and sesquiterpines**, which are hydrocarbons with the general formula $(C_5H_8)_n$.
- The elevated concentration of **α-pinene** in *S. cumini* leaves oil makes it potentially useful in medicines because this display antibacterial, antifungal, anti-inflammatory, insecticidal and antioxidant activity, and it is used traditionally as flavoring agent and antimicrobial material in food (Mohamed et al, 2013).
- Compounds of leaves, barks, fruits, stems, and roots contribute to the air cleaning and free from bacteria (Forbey et al 2009).

7. **Champa (*Michelia champaca* L.)**

   Champa is well known plant for its fragrance. But somewhere it has antibacterial properties. Its leaves and flowers showed the presence of Alkaloids, *tannins, glycosides, carbohydrates, amino acids, flavonoids and sterols.*

   These componants are responsible for *Antibacterial and free* radical scavenging activity of *Michelia champaca* L.(Ananthi and Chitra 2013).

   - **Volatile oil - β-elemene, β-caryophyllene, α-humulene, β-selinene, and α-cadinol (Lago et al, 2009).**
   - **β-caryophyllene** have been reported as an antimicrobial agent from different plants (Dahham et al, 2015).

8. **Hajara (*Tagetes erecta*):**

- Preliminary phytochemical analysis with *Tagetes erecta* proved that the plant is highly rich in alkaloids, phenolic compounds, flavanoids, salicylic acid, terpenes etc.
- The chief Volatile Oil components of Arial parts were reported as **cis-ocimene** (18.46%), **(E)-oscimene** (8.65%), **l-limonene** (11.16%), (**E)-tagetone** (10.56%), **β-caryophyllene** (6.9%) and **dl-limonene** (4.16%) with their Antimicrobial activity (Tripathi et al, 2012).

Chemical Composition status in 3 stages of Dhoopbatti (Solid Form, Fumes and Ash)

For Mixture- I Herbal Gau Dhoopbatti - Cow Dung, Cow Urine, Neem, Arujna, Jamun and Bhadraksh

Ingredients	Major Photochemistry	During Fume development Active compounds (Responsible for Antibacterial activity)	Contents of ash (Responsible for Antibacterial activity)
Cow dung	• Organic material 14.6 (%) • Total nitrogen 0.30–0.45 (%) • Total phosphorus 0.15–0.25 (%) • Total potassium 0.05–0.15 (%) • Flavanoids, • Glycosides, • teroids, • Tannins, • Phenols, Humic acid	Phenols	elements differ in the subsequent decreasing order of concentration; K>Na>Mg>Ca>Fe>Al>Zn
Cow Urine	• Urea • uric acid • Creatinine • Aurum hydroxide	Creatinine	
Neem	• Azadirachtin	• γ-Elemene (24.06%) • 3,7 (11)-eudesmadiene(6.83%) • caryophyllene(6.40%) 10s,11s-himachala-3(12),4-diene (6.36%)	
Arjuna	• tannins, • terpenoids, • alkaloids • glycosides, • flvanoids and • phenols	• carvacrol (11.17%), • thymol (6.52%), • α-terpinyl acetate (5.92%) • anethole (5.13%)	
Jamun	• flavonoids	• Phenols • Alkaloids • α-pinene- Volatile Oil	
Bhadraksh	• octacosanol, • taraxeroloac, • friedelin-3- áoac, â-sitosterol, and Friedelinol-3-acetate, • tartaric acid or tannins such as catechins, colatannins and caffeine, kaempferol , friedelin, betulin, â-sitosterol	• precocene I (56.0%), • P-caryophyllene (13.7%)	

For Mixture- II Herbal Gau Dhoopbatti - Cow Dung, Cow Urine, Neem, Champa, Hajara and Bhadraksh

Ingrdients	**Major Photochemistry**	**During Fume development Active compounds (Responsible for Antibacterial activity)**	**Contents of ash (Responsible for Antibacterial activity)**
Cow dung	• Organic material 14.6 (%) • Total nitrogen 0.30–0.45 (%) • Total phosphorus 0.15–0.25 (%) • Total potassium 0.05–0.15 (%) • Flavanoids, • Glycosides, • steroids, • Tannins, • Phenols	Phenols	elements differ in the subsequent decreasing order of concentration; K>Na>Mg>Ca>Fe>Al>Zn
Cow Urine	• Urea • uric acid • Creatinine • Aurum hydroxide	Creatinine	-
Neem	• Azadirachtin	• γ-Elemene (24.06%) • 3,7 (11)-eudesmadiene(6.83%) • caryophyllene(6.40%) 10s,11s-himachala-3(12),4-diene (6.36%)	-
Champa	• Alkaloids • *tannins,* • *glycosides,* • *carbohydrates,* • *amino acids,* • *flavonoids and sterols.*	• β-caryophyllene	-
Hajara	• alkaloids, • phenolic compounds, • flavanoids, • salicylic acid, • terpenes etc.	• cis-ocimene (18.46%), • (E)-oscimene (8.65%), • l-limonene (11.16%), • (E)-tagetone (10.56%), • β-caryophyllene (6.9%) • dl-limonene (4.16%)	-
Jamun	• flavonoids	• Phenols • Alkaloids • α-pinene- Volatile Oil	-
Bhadraksh	• octacosanol, • taraxeroloac, • friedelin-3- áoac, â-sitosterol, and Friedelinol-3-acetate, • tartaric acid or tannins such as catechins, colatannins and caffeine, kaempferol , friedelin, betulin, â-sitosterol	• precocene I (56.0%), • P-caryophyllene (13.7%)	

Air cleansing Analysis of Herbal Gau Dhoopbatti; Morphological and Biochemical analysis of Air Born Bacterial strains-

1. **Air cleansing Analysis of Herbal Gau Dhoopbatti**
    - Nutrient agar Agar Plates were prepared and exposed to various environments (Microbiology Lab, Garbage area, Faculty Room, Jayoti Vidyapeeth women's University, Jaipur) in triplicates for control and Mixture I and II (Table-3).
    - Plates (For Mixture I and II) except control plate were exposed to the prepared Dhoopbatti I (Mixture I) and Dhoopbatti (Mixture II) for 30 minute respectively in a sterilized chamber (Figure-2; Table-4). After 24 hours of incubation at 37^0C for Nutrient agar plates, Bacterial growth was observed.

Sr. No	Nutrient Agar Plates Time of exposure	Time of exposure
1	Microbiology lab	10 Minute
2	Garbage area	10 Minute
3	Faculty Room	10 Minute

Table – 3 Details of Exposure area and Time duration for Microbial Collection (Area selected at Jayoti Vidyapeeth Women's University, Jaipur)

Sr. No	Nutrient Agar Plates Time of exposure	Exposure of Dhoopbatti	Time of exposure (LAF Chamber) (3 x 2 x 2 ft)
1	Pattriplate set I	No Exposure	30 Minute
2	Pattriplate set II	Dhoopbatti I	30 Minute
3	Pattriplate set III	Dhoopbatti II	30 Minute

Table – 4 Details of Exposure time and Dhoopbatti Type

Figure – 2 Evaluation of cleansing activity of prepared Herbal Gau dhoopbatti (performed in Closed Chamber of Laminar Air Flow, Microbiology Lab, JVWU, Jaipur)

A survey was also conducted at Campus of Jayoti Vidyapeeth Women's University, Jaipur to evaluate the acceptability of the Herbal Gau dhoopbatti. Different parameters such as smell, appearance, smoke and comparison with active formulations were examined.

2. Morphological and Biochemical analysis of Air Born Bacterial strains-

2.1 Preparation of pure bacterial strains

Materials

- Nutrient Agar
- Distilled water
- Slants
- Conical flask
- Culture

Procedure

- Nutrient media was prepared and autoclaved at 121^0c for 15-20 min.
- Media was poured in the sterile slants.
- Any single colony was picked up from the bacterial cultures with the help of loop and inoculates in to the slants.
- Then the slants were kept at 37^0c of incubation period for the growth of bacteria.
- The slants containing single type of colony were used further for the characterization of bacteria.

2.2 Identification of the bacteria isolated

The bacterial strains were identified by performing certain tests.

Materials

- Bunsen burner
- Slides
- Cover slip
- Microscope
- Inoculating loop
- **Microscopic identification**

➢ Digital Microscopic identification was performed at Innovation Center of Jayoti Vidyapeeth Women's University, Jaipur

➢ The inoculating loop was heat on the Bunsen burner till the red hot and allows it to cool for 1-2 min.

➢ Place 1-2 drops of distil water on the slide and then take some growth from the Petri plates from the inoculating loop on the slide.

➢ Place the cover slip on it and observe under light microscope.

➢ This procedure was repeated for all the cultures of the bacteria and identifies the bacteria by their morphological characters.

➢ Finally the bacterial strains were identified from the Sample of Air collected samples of Garbadge, Microbiology Lab and Faculty Room.

- **Gram Reaction Test**

➢ Place a one drop of distilled water on the slide and transfer the small colony of the bacteria by inoculating loop and make the smear.

➢ Add 1-2 drops of crystal violet for few min. & then wash it with tap water.

➢ Add 1-2 drops of iodine solution for few min. and wash it with tap water.

➢ Add 1-2 drops of ethanol for few min. and wash it with tap water

➢ Similarly add some drops of safranin for few min. and then wash it.

➢ Gram positive bacteria stains purple and Gram negative stains red/pink.

- **Catalase Test**

➢ Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick

- Place a drop of 3% H_2O_2 on to the slide and mix.
- A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling.
- A negative result is no bubbles or only a few scattered bubbles.

RESULTS

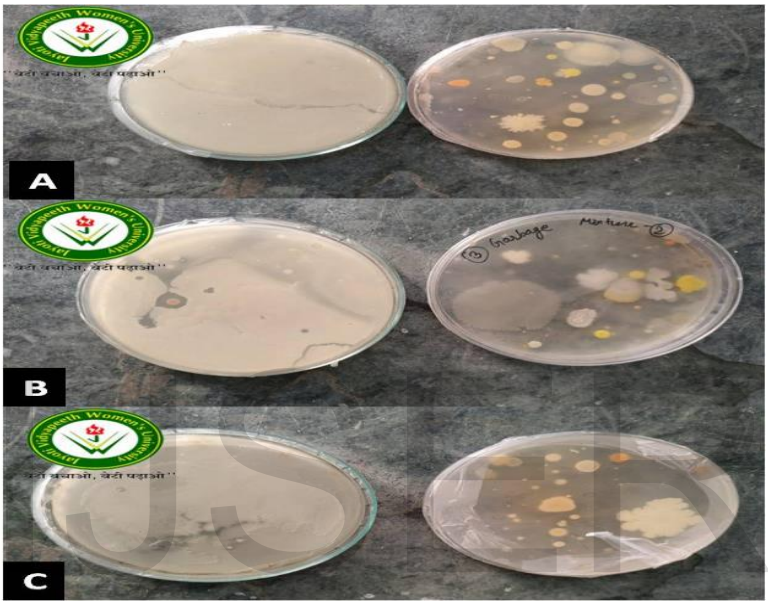

Figure- 3 Antibacterial activity of Dhoopbatti- I (Mixture-I) with Exposure area of (A) Microbiology Lab (B) Garbage Area (C) Faculty Room, JVWU, Jaipur. Left side pattriplate- Control, Right Side pattriplate- Exposed to Herbal Gau Dhoopbatti-I (Mixture-I)

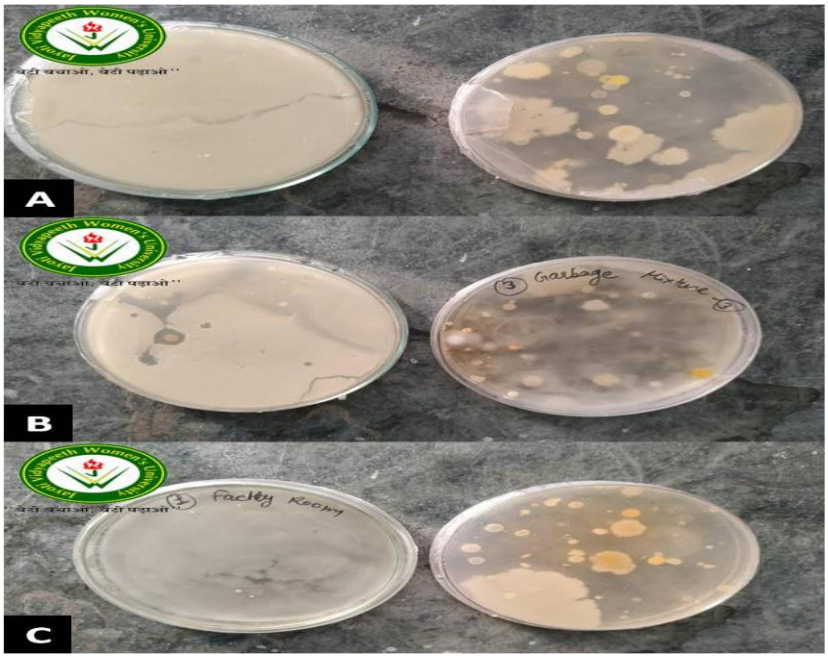

Figure- 4 Antibacterial activity of Dhoopbatti- II (Mixture-II) with Exposure area of (A) Microbiology Lab (B) Garbage Area (C) Faculty Room, JVWU, Jaipur. Left side pattriplate- Control, Right Side pattriplate- Exposed to Herbal Gau Dhoopbatti-II (Mixture-II)

Sr. No	Plates exposed to	No of Bacterial colonies without exposure to dhoopbatti	No of Bacterial colonies with exposure to dhoopbatti	
			With exposure of Dhoopbatti I (Mixture I)	With exposure of Dhoopbatti II (Mixture II)
1	Microbiology Lab	uncountable	23	27
2	Garbage area	uncountable	25	25
3	Faculty Room	uncountable	22	26

Table-5 Statistical Analysis of Bacterial study

- From Figure 3 and 4 it was observed that on exposure of pattriplates with Herbal Gau Dhoopbattti I & II respectively, the aerial Bacteria was inhibited.
- Colony counting number was also observed in decreasing manner in both type of exposure of Herbal Gau Dhoopbatti (Table-5).

- In control, colony counting was uncountable. On the other hand, 23, 25 and 22 colony number were counted for Microbiology Lab, Garbage area and Faculty room, JVWU, Jaipur with exposure of Dhoopbatti- I (Mixture –I).
- While almost similar results were found for exsposure of Herbal Gau Dhoopbatti- II. It was 27, 25 and 26 colony number for Microbiology Lab, Garbage area and Faculty room.

Survey analysis of acceptability-

- Both type of dhoopbatti were mostly acceptable for all aspects.
- Dhoopbatti- II was highly appreciable for its fragrance due to use of Champa and hajara flower and leaves.
- 100 % acceptability was recorded for both types of Herbal Gau Dhoopbatti for use because of having antibacterial activity (Table- 6).

Questions	**Percentage of Acceptibility**	
	Dhoopbatti I (Mixture- I)	**Dhoopbatti II (Mixture- II)**
Smell of the dhoopbatti appreciable?	80%	93.33%
Fragrant smell (such as floral and perfumes)	73.33%	93.33%
Smoke irritates to eyes?	60%	80%
Appearance acceptable?	80%	80%
Would like to use it in their surrounding area?	80%	80%
Would advise it for use if established to have antibacterial activity?	100%	100%
Is it better than the dhoopbatti formulations accessible in the market?	93.33%	93.33%

Table 6: Survey of evaluation at Campus of JVWU, Jaipur for acceptability of the prepared Herbal Gau dhoopbatti

Isolation of the bacteria-

From bacterial culture plate, Four pure culture (Strain A- Yellow Color, B- Ceamy White, C- White and D – Orange) were isolated. They were identified by microscopic morphological analysis, Biochemical analysis and Gram staining

Strain	Colour on NA	Shape	Gram reaction	Catalase test	Possible Bacterial strains
A	yellow	spherical (cocci), and form in grape-like clusters	Positive	positive	***Staphylococcus sp.***
B	Creamy White	Coccus	Positive	Negative	***Streptococcus sp.***
C	White	Rods in chain	Positive	positive	***Bacillus sp.***
D	Orange	Rode shaped	Negative	-	***Serratia Sp.***

Table- 7 Morphological and biochemical characteristics of test strains

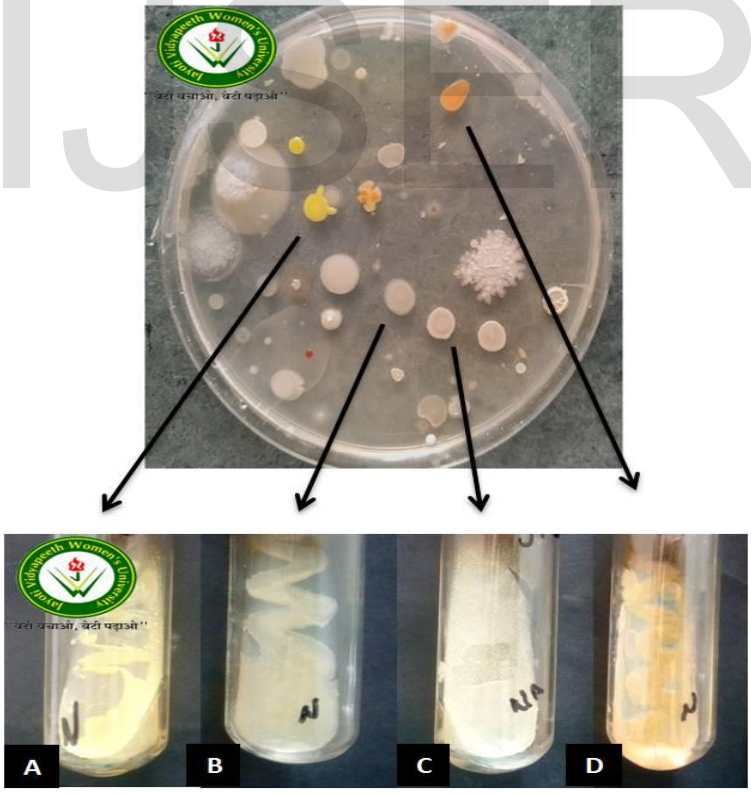

Figure- 5 : Pure culture of Bacterial Strains

- Possible four pur air borne bacterial strains were identified on the basis of Morphological and Biochemical analysis. These possible strains are ***Staphylococcus sp., Streptococcus sp., Bacillus sp.and Serratia Sp.***
- Gram positive bacteria were ***Staphylococcus sp., Streptococcus sp., Bacillus sp.***
- Gram Negative bacteria was reported- ***Serratia Sp.***

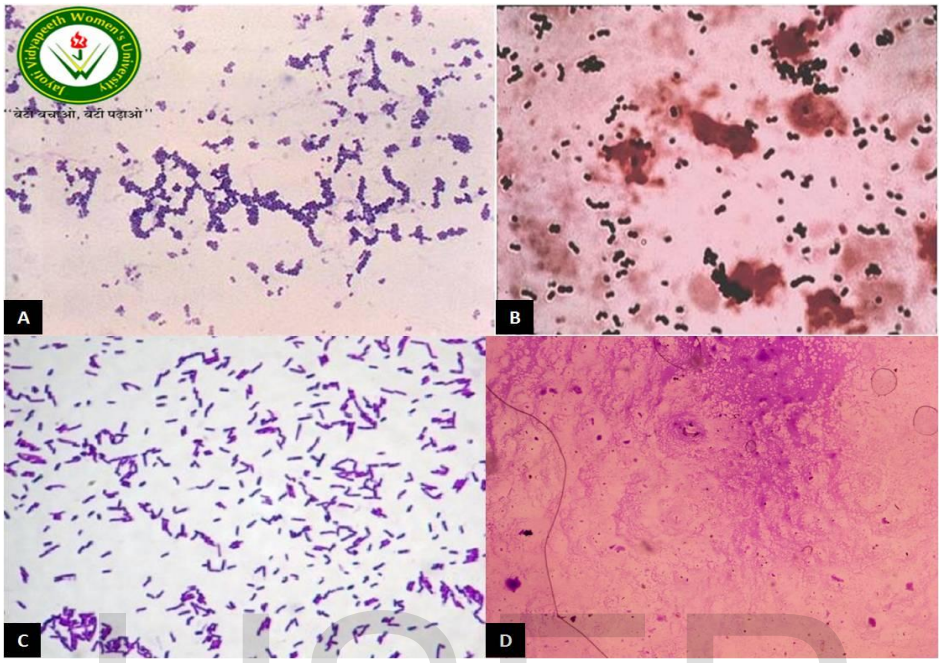

Figure- 6 Microscopic identification by Gram reaction test at Innovation Center of JVWU, Jaipur

DISCUSSION-

The efficiency of these Herbal Gau dhoopbatti could be attributed to the mixture of cow dung, cow urine, Medicinal Plants Mixtures. The medicinal properties of cow dung has been reported to formulate drugs for several diseases caused by antibiotic resistant pathogenic microorganisms (Reddy et al, 2013; Rajeswari et al, 2016). Antimicrobial Activities of Cow Urine also has been suggested Against Various Bacterial Strains due to its active components (Ahuja et al, 2012). Some reports have been revealed that leaf extract of Neem combination has maximum inhibition results on *E. coli, Staphylococcus aureus, Pseudomonas aureginosa, Bacillus subtilis* (Parashar et al, 2018). The antimicrobial effectiveness of the polar extracts of Arjuna was reported to be proportionate with high polyphenol content (Debnath et al, 2013; Dalei and Sahoo, 2016). Bhadraksha (*Guazuma ulmifolia* Lam.) L poses precocene I (56.0%), P-caryophyllene (13.7%) and (2Z,bE)-farnesol (6.6%) (Adaga et al, 1997). Precocene I and P- caryophyllene has reported antimicrobial activity. Some reports also suggested about Antibacterial activity of Jamun leaf extracts against multidrug resistant pathogenic bacteria

(Imran et al, 2017). Champa and Hajara also showed antibacterial activity against Pathogenic bacteria (Topp et al, 1998; Ananthi and Chitra 2013).

A variety of dhoops are accessible in the market having coal, chemicals, incense, and saw dust, which causes pollution in the air. The proposed Dhoopbatti products are pure natural and are free from all harmful materials. Therefore with the use of cow products and a mixture of herbal plant powders, an effort was ready to expand an tremendously practicable and simple to use herbal formulation which can inhibit the pathogenic bacterial growth and minimize the use of potentially harmful chemicals.

OBSERVATION AND FUTURE SCOPE-

- Both Mixture showed Air cleansing Activity and Inhibit Air borne Bacterial growth.
- Survey analysis for acceptability is also in favour of both Herbal Gau Dhoopbatti because of its antibacterial activity.
- With all these qualities, Herbal Gau Dhoopbatti can be use as Natural Room purifier.
- Ingredients for Mixture I & II Dhoopbatti have all natural gradients are rich with Phytochemicals. That's why they are safe and secure to use.
- Ingredients of both types also have some volatile compounds such as phenols, volatile oils which have antibacterial activity.
- During the development of fumes (burning of dhoopbatti), these volatile compounds start to spread in nearby area. As these volatile compounds have antibacterial activity (Bacteriostatic quality), they start to inhibit the growth of air borne bacterial strains. Thats how it may work as Air cleansing factor.
- On the other hand, remaining ash content of both dhoopbatti is also useful and can be used as disinfectant for cleaning utensils. Because dhoopbatti's one major component is Cow dung. Ash content of cow dung has been reported pure components in range of K>Na>Mg>Ca>Fe>Al>Zn. These compounds have antibacterial activity.
- This report suggests that for normal room, 1 Dhoopbatti is required to use during intervals of 2-3 Hours. If Room area is open for entry- exit, then 1 Dhoopbatti is required with interval of 1 Hour.
- Ash of Dhoopbatti is also useful due to cow dung components. It can be used to clean daily basis utensils due to its Antibacterial activity.

- Ingredients Cow Dung, Neem and Arjuna have reported Antiviral activity also. So this report also suggests that as it is Antibacterial as well as it can help in controlling Viral infection also. This study opens future scope to find out this formulation's capability to inhibit Viral strains.

CONCLUSION

This study revealed about the evaluation of Herbal Gau Dhoopbatti formulations for air cleansing and suggest remaining ash as disinfectant for cleaning utensils. Through positive results of the study, it is evident that Herbal Gau Dhoopbatti formulations can cleanse the air and inhibit the growth of bacteria. Herbal Gau Dhoopbatti formulations are having all natural sources having medicinal properties and Antibacterial properties as well. The current study revealed that Herbal Gau Dhoopbatti formulations can reduce the pathogenic airborne bacteria from air and can maintain its effect till 2-3 hours for a room. Ash of Herbal Gau Dhoopbatti can be used as disinfectant for cleaning utensils and wash hands rarely. Because ash also has some antimicrobial agents. Herbal Gau Dhoopbatti having distinct superiority and it is formulated from affordable sources. It creates an optimistic surroundings and can perform as a room purifier and disinfectant. Herbal Gau Dhoopbatti preparation is the initiative by the University to support community to offer affordable natural products in concern of health.

REFERENCES

1. Adaga AMC et al, (1997) Volatile Constituents from Leaves of Guazuma ulmifolia Lam. J. Essent. Oil Res., 9, 705-706
2. Ahuja A, Kumar P, Verma A,Tanwar R S (2012). Antimicrobial Activities of Cow Urine Against Various Bacterial Strains. Int J Recent Adv Pharm Res. 2(2):84-87.
3. Amalraj A and Gopi S (2017). Medicinal properties of *Terminalia arjuna (Roxb.) Wight & Arn.*: A review. J Tradit Complement Med. 7(1): 65–78.
4. Amit N., Nilesh M., Sunanda B., Salunke Amru 5gomutra (cow urine): a multidimensional drug review article.
5. Ananthi T, Chitra M. (2013). Screening of in-vitro anti-inflammatory activity of Michelia champaca Linn. Flowers. Asian Journal of Pharmaceutical and Clinical Research. 6(5):71-72.

6. Aneja K R, Sharma C, Joshi R (2012). Antimicrobial activity of Terminalia arjuna Wight & Arn.: An ethnomedicinal plant against pathogens causing ear infection. Braz J Otorhinolaryngol.78(1):68-74
7. Bano S et al. (2019). Comparative analysis of oil composition and antibacterial activity of aerial parts of *Terminalia arjuna* (Roxb.). Formerly Natural products letters. https://doi.org/10.1080/14786419.2018.1557656
8. Barot N.S. and Bagla H.K. (2009) Extraction of humic acid from biological matrix dry cow dung powder. Green Chemistry Letters and Reviews Vol. 2, No. 4, 217221
9. Chatha S A S, Hussain A I, Asad R, Majeed M and Aslam N (2014). Bioactive Components and Antioxidant Properties of Terminalia arjuna L. Extracts J Food Process Technol. 5:2
10. Clean Air Act. https://www.epa.gov/sites/production/files/201508/documents/peg.pdf. April 2007.
11. Cleaning and the Environment. https://www.ciriscience.org/a_269-Cleaning-and-theEnvironment. 8 February, 2012
12. Dalei J and Sahoo D (2016). Evaluation Of Antimicrobial Activity And Phytochemical Screening Of Epicarp And Endocarp Parts Of Elaeocarpus Ganitrus. Int J Pharm Bio Sci . 7(2): (P) 265 - 269
13. Debnath S , Dey D, Hazra S , Ghosh S , Ray R , Hazra B (2013). Antibacterial and antifungal activity of Terminalia arjuna Wight & Arn. bark against multi-drug resistant clinical isolates. Journal of Coastal Life Medicine. 1(4): 312-318.
14. Devenport NA, Blenkhorn DJ, Weston DJ, Reynolds JC,and Creaser CS (2014) Direct Determination of Urinary Creatinine by Reactive-Thermal Desorption-Extractive Electrospray-Ion Mobility-Tandem Mass Spectrometry. Anal Chem. 2014 Jan 7; 86(1): 357–361.
15. Forbey JS, Harvey AL, Huffman MA et al (2009) Exploitation of secondary metabolites by animals: a response to homeostatic challenges. Integr Comp Biol 49:314–328.
16. Gupta D and Kumar M (2017). Evaluation of *in vitro* antimicrobial potential and GC–MS analysis of *Camellia sinensis* and *Terminalia arjuna*. Biotechnol Rep (Amst). 13: 19–25.

17. Imran M, Imran M , Khan S (2017). Antibacterial activity of Syzigium cumini leaf extracts against multidrug resistant pathogenic bacteria. Journal of Applied Pharmaceutical Science. 7 (03):168-174.

18. Kumar NA and Gurunani SG (2019) Guazuma ulmifolia LAM: A review for future view. Journal of Medicinal Plants Studies. 7(2): 205-210.
19. Kumar NA and Gurunani SG Guazuma ulmifolia LAM: A review for future view. Journal of Medicinal Plants Studies 2019; 7(2): 205-210.
20. Pachori RR, Kulkarni NS, Sadar PS, Mahajan NN (2013). Effect of agnihotra fumes on aeromicroflora. The Bioscan. 8(1):127-129.
21. Parashar G, Sutar N and Sanap S (2018). Antibacterial Activity Of Mixture Of Leaf Extracts Of Neem (*Azadirachta Indica* Linn.) And Tantani (*Lantana Camara*). , IJPSR. 9(6): 2545-2549.
22. Perera A.G.W.U., . Karunaratne M.M.S.C, Chinthaka S.D.M. (2018). Bioactivity and Volatile Profiling of Azadirachta indica Leaves for the Management of Maize Weevil, Sitophilus zeamais (Motsch.) Infestations. Journal of Tropical Forestry and Environment. 8(01): 10-24.
23. Rajeswari S., Poongothai E. , Hemalatha N. (2016). Antimicrobial Activites Of Cow Dung Extracts Against Human Pathogens. Int J Curr Pharm Res. Vol 8:4, 9-12.
24. Reddy R R, Kumari K, Lokanatha , Mamatha , Reddy D (2013). Antimicrobial activity of Azadirachta Indica (neem) leaf, bark and seed extracts. Int. J. Res. Phytochem. Pharmacol., 3(1), 1-4.
25. Shah, C.P., Patel, D.M., Dhami, P.D., Kakadia, J., Bhavsar, D., Vachhani, U.D., et al. (2011) *In vitro* screening of antibacterial activity of cow urine against pathogenic human bacterial strains. Int J Curr Pharm Res. 3:91–2.
26. Tiwari V, Darmani NA, Yue BYJT, and Shukla D. *In vitro* antiviral activity of neem (*Azardirachta indica* L.) bark extract against herpes simplex virus type-1 infection. Phytother Res. 2010 Aug; 24(8): 1132–1140.
27. Topp E. , Millar S. , Bork H. , Welsh M. (1998). Effects of marigold (*Tagetes* sp.) roots on soil microorganisms Biol Fertil Soils. 27 (2): 149-154

28. Tripathi B, Bhatia R, Walia S, Kumar B(2012) Chemical Composition and Evaluation of Tagetes erecta (Var. Pusa Narangi Genda) Essential Oil for its Antioxidant and Antimicrobial Activity . Biopestic. Int. 8(2): 000-000 (2012).

29. Why Should You Be Concerned About Air Pollution? http://www.epa.gov/airquality/peg_caa/concern.html. April 2007.

30.

31.