Research Article

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PHYTOCHEMICAL AND ANTIMICROBIAL ACTIVITY OF AQUEOUS, ALCOHOLIC AND VOLATILE EXTRACT OF DUPANA DRAVYAS

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ABSTRACT

Dupana dravyas (Herbal fumigation drug) are combination of six herbal medicines such as Garlic (*Allium sativum)*, Tulsi *(Ocimum tenuiflorum*), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa),* Mustard (*Brassica juncea),* Curry leaves (*Murraya koenigii).* Altogether in an equal quantity were crushed and made paste to prepare a small ball and dried in the sun shade. This can be used as an herbal fumigant for sterilization of hospital environment. This preparation has been subjected to Phytochemical and antimicrobial activity of maceration extract of aqueous, alcoholic and volatile extract of Dupana dravyas. Antibacterial activity of different concentrations of drugs was tested against air isolated bacteria out of petri-plate exposure technique by cup diffusion method. It was observed that the Dupana dravyas significantly inhibited all the isolates isolated from air. The present study is therefore successful in indicating that these Dupana dravyas are useful in controlling the airborne pathogens which causes diseases associated with airborne nosocomial infections.

Keywords: Dupana dravyas, Volatile extract, petri-plate exposure, nosocomial

INTRODUCTION

Germicidal, antimicrobial, anti-helminthic property of Garlic (*Allium sativum)*, Tulsi *(Ocimum tenuiflorum*), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa),* Mustard (*Brassica juncea),* Curry leaves (*Murraya koenigii)* are explained in detailed in Ayurveda classics[1] and also lots of studies have proven them. All these are natural and easily available common herbs. Presence of antimicrobial principles such tannins, glycosides, alkaloids, flavonoids, saponins, anthraquinones, cardiac glycoside, steroidal ring, steroidal trepans and carbohydrates at different concentrations were highlighted by phytochemical analysis of fresh and dried leaf extracts of *Ocimum gratissimum*[2].

Nature has provided a complete store house of remedies to cure all ailments of mankind. The natural or herbal remedies are still the backbone of medicines. Phyto-therapy is a medicinal practice based on the use of herbal plants and their extracts. These herbs or plants and their active ingredients are used in traditional herbal remedies. The easy availability, low cost and negligible side effects, natural products are popular in the now a days in the world[3]. All the herbs produced bewildering variety of phytochemical like primary metabolites [carbohydrates, fats, proteins] and secondary metabolites (Alkaloids, flavonoids, steroids, saponins and polyphenols) for their normal metabolic activities[4]. These secondary metabolites showed various biological activities and act in plant defence mechanisms. The chemical profile of a single plant may vary over time as it reacts to changing conditions. The secondary metabolites have therapeutic actions, which produced drugs[5]. Varity of drug and secondary metabolites can be obtained by various medicinal plants, about 80% of individuals from developed countries use traditional medicine[6]. Phytochemical study will reveal their properties, safety and efficiency. Medicinal plants have been utilised for years in daily life to treat disease all over the world[7].These medicinal plants and secondary metabolites used as a source of potent and powerful drugs[8]. There has been a revival of interest in herbal medicines.

MATERIALS AND METODS

Phytochemical study

Alkaloid, Saponins, Flavonoids Anthra-quinones, tannins, steroids, phenols and cardiac glycoside were quantitatively determined by adopting the Standard procedure[12].

Aqueous and Alcoholic Extraction by Maceration

The Dupana dravyas (Herbal fumigation drug) which are used in the procedure were subjected to the extraction procedure soaking the 25gm of crude drug, in an 250ml of distilled water and ethanol respectively for about 7 days in a shaker after 7 days filtrate were kept in an water bath in 60º C up to water evaporate to get the thick extract of dupana[11].

Volatile oil

Garlic (*Allium sativum)*, Tulsi *(Ocimum tenuiflorum*), Neem (*Azadirachta indica*), Turmeric (*Curcuma longa)* and Mustard (*Brassica juncea),* Curry leaves (*Murraya koenigii)* Volatile oils were procured from Sri Venkatraman industries, Talaya Chauki, Kannauj-209725(U.P.) India. ISO9001:2008Certified.
The Aqueous alcoholic extraction and volatile organic compounds obtained from the Dupana dravyas were subjected to Antimicrobial sensitivity test by well diffusion method, Muller Hinton Agar plate were swabbed with standard McFarland inoculums Replace the lid of the dish leave it for 5 minutes. Make 6 equidistant wells on the plates with the help of sterile cork borer. Add 100 µl of the control (sterile distilled water) and Dupana dravya extracts of different concentration (16µg, 8µg, 4µ.2µg and 1µg) onto the labelled wells. Incubate

all the plates at 37 ºC for 24 hours. After incubation period, the zones of inhibition were measured with a ruler.

RESULTS

Phytochemical and Aqueous, alcoholic and volatile extract of Dupana dravya procedures were carried out as per standard protocol and results were tabulated below(Table-1), extract were treated against pathogenic bacteria to see the antimicrobial activity against *E.coli.*, *Streptobacilli* spp., *Staphylococci* spp. and *Pneumococci* spp. Isolated from Hospital environment. Results obtained are tabulated below (Table 2 to 6).

DISCUSSION

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of dupana dravyas that serves as defense mechanism against prediction by many microorganisms, insects and other herbivores. Analysis of plant extracts revealed the presence of coumarins, flavonoids, glycosides, phenols, saponins, steroids and tannins in most of the selected plants which could be responsible for the observed antimicrobial property.

Table 1: Preliminary phytochemical screening of flakes of dried herbal paste

Tests	Colour if positive	Result
Alkaloids		
Dragendorff's test	Orange precipitate	Orange precipitate
Wagner's test	Red precipitate	Red precipitate
Mayer's test	Dull white precipitate	Dull white precipitate
Hager's test	Yellow precipitate	Yellow precipitate
Steroids		
Liebermann- Burchard test	Bluish green	Bluish green
Salkowski test	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer	Bluish red to cherry red color in chloroform layer and green fluorescence in acid layer
Carbohydrate		
Molisch test	Violet ring	Violet ring
Fehling's test	Brick red precipitate	Brick red precipitate
Benedicts test	Red precipitate	Red precipitate
Tannin		
With $FeCl_3$	Dark blue or green or brown	Brown
Flavonoids		
Shinoda's test	Red or pink	Green color
Saponins		
With $NaHCO_3$	Stable froth	No stable froth
Triterpenoids		
Tin and thionyl chloride test	Pink	Pink
Coumarins		
With 2 N NaOH	Yellow	Yellow
Phenols		
With alcoholic ferric chloride	Blue to blue black, brown	Brown color
Carboxylic acid		
With water and $NaHCO_3$	Brisk effervescence	No brisk effervescence
Amino acid		
With ninhydrine reagent	Purple colour	No purple colour
Resin		
With aqueous acetone	Turbidity	No turbidity
Quinone		
5% NaOH	Pink/purple/red	Red color

Test	Inference
Alkaloid	+
Steroid	+
Carbohydrate	+
Tannin	+
Flavonoids	-
Saponins	-
Terpenoid	+
Coumarins	+
Phenol	+
Carboxylic acid	-
Amino acids	-
Resins	-
Quinone	+

Table 2: Antibacterial activity against *E. coli*

Organism	Concentration	Aqueous extract zone of inhibition in mm	Alcoholic extract zone of inhibition in mm	Volatile Extract zone of inhibition in mm
E. coli	16µg	18	18	22
	8 µg	20	22	22
	4 µg	22	22	24
	2 µg	22	24	24
	1 µg	24	24	24
	Control(D.W)	G	G	G

(Note: µg–micro gram, D.W- distilled water, G – Growth seen, mm- millimetre)

Table 3: Antibacterial activity against *Pseudomonas* spp

Organism	Concentration	Aqueous extract zone of inhibition in mm	Alcoholic extract zone of inhibition in mm	Volatile Extract zone of inhibition in mm
***Pseudomonas* sps**	16µg	16	18	18
	8µg	16	18	18
	4µg	18	20	20
	2µg	18	22	20
	1µg	20	22	22
	Control(D.W)	G	G	G

(Note: µg- micro gram, D.W- distilled water, G – Growth seen, mm- millimetre)

Table 4: Antibacterial activity against *Streptobacilli* spp

Organism	Concentration	Aqueous extract zone of inhibition in mm	Alcoholic extract zone of inhibition in mm	Volatile Extract zone of inhibition in mm
***Streptobacilli*sps**	16µg	20	22	20
	8µg	20	22	20
	4µg	22	24	22
	2µg	22	24	22
	1µg	24	24	24
	Control(D.W)	G	G	G

(Note: µg - micro gram, D.W- distilled water, G – Growth seen, mm- millimetre

Table 5: Antibacterial activity against *Staphylococci* spp

Organism	Concentration	Aqueous extract zone of inhibition in mm	Alcoholic extract zone of inhibition in mm	Volatile Extract zone of inhibition in mm
***Staphylococci* sps**	16µg	18	20	16
	8µg	18	20	16
	4µg	18	20	18
	2µg	20	22	20
	1µg	22	22	22
	Control(D.W)	G	G	G

(Note: µg - micro gram, D.W- distilled water, G – Growth seen, mm- millimetre)

Table 6: Antibacterial activity against *Pneumococci* spp.

Organism	Concentration	Aqueous extract zone of inhibition in mm	Alcoholic Extract zone of inhibition in mm	Volatile Extract zone of inhibition in mm
***Pneumococci* sp.**	16µg	16	18	18
	8µg	16	18	18
	4µg	18	20	20
	2µg	20	22	22
	1µg	22	24	22
	Control(D.W)	G	G	G

(Note: µg - micro gram, D.W- distilled water, G – Growth seen, mm- milli meter)

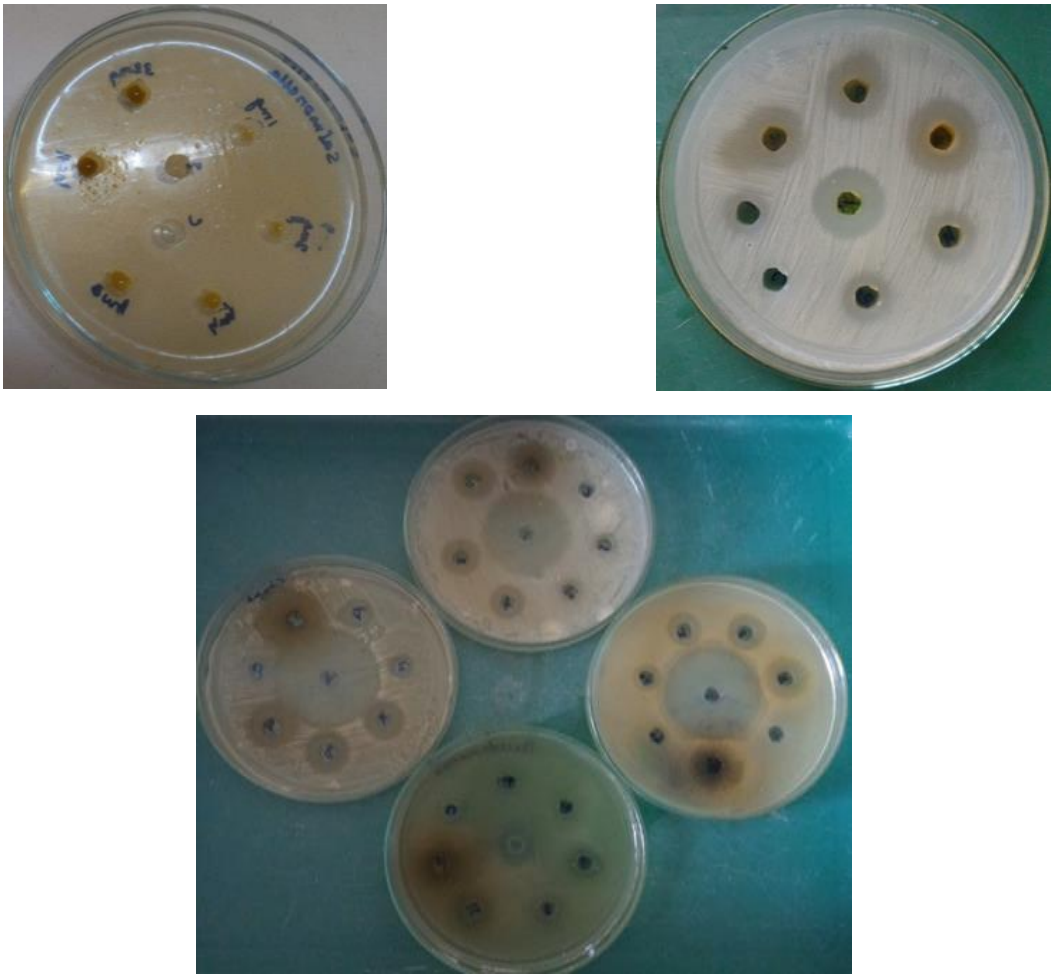

Figure 1: Antimicrobial activity of Aqueous, Alcoholic and Volatile Extract of Dupana Dravyas

Dupana dravyas (Herbal fumigation drug) were showing highly significant zone of inhibition against *E.coli.*, *Streptobacilli* spp., *Staphylococci* spp. and *Pneumococci* spp. In 2µg and 1µg concentration. This study is genuine work carried out to see the antimicrobial activity of combination of six dupana dravyas, related studies were not yet done on this work. Dhupana dravyas are effective against air borne microorganisms. Antimicrobial property against bacterial species has been proved by petriplate exposed plates by before and after fumigation, by reduction of growth in the HiMedia plates. They have been non irritant and did not disturb any individual or any individual's routine activity. There was no adverse reaction to eye, skin, and respiratory system to any individual during the procedure[10].

CONCLUSION

Airborne particles are a major cause of respiratory ailments of humans, causing allergies, asthma, and pathogenic infections of the respiratory tract. The present research work concludes that Dupana dravyas are medicaly important with varied pharmacological spectrum. The Dupana dravyas shows the presence of many phytochemical constituents which are responsible for varied pharmacological and medicinal property. The evaluation needs to be carried out on Dupana dravyas in order to uses and formulation of the drugs in their practical clinical applications, which can be used for the welfare of the mankind

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