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Antimicrobial Potential of Panchagavya Formulation from Indian Cow Breeds

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ABSTRACT

The Sanskrit word panchagavya means the "mixture of five cow products", viz. dung, urine, milk, curd and ghee. The present study involves standardization of method for preparation of panchagavya formulations on the basis of antimicrobial activity. For that, panchagavya prepared in three different ratios (A, B and C) were kept for fermentation at two different temperatures (37° and 25°C) up to 30 days. At 10 days interval, raw samples and their distillates were analyzed for pH and antimicrobial activities against selected indicator strains. The ratio A, B and C had pH value 7.49±0.09, 8.01±0.16 and 8.23±0.26, respectively at 0 day while distillate of corresponding samples showed pH towards the alkaline side. On fermentation the pH value of RA and its distillate shifted towards acidic side while that of ratio B and C and their distillate towards alkaline side. No antibacterial activity was observed against gram negative bacteria. The distillate of ratio A (DRA) fermented at 37°C showed maximum activity against the two-gram positive bacteria i.e.,*Bacillus. subtilis* and *Bacillus. cereus* on 20th day. Similarly in antifungal activity, the distillate of ratio A (DRA) fermented at 37°C showed maximum activity against *Candida. Butyri, Rhodotorulaglutinis, Penicillium camemberti* and *Aspergillus niger*. Overall, the highest antimicrobial activity was observed in the distillate of panchagavya prepared by mixing all the raw ingredients in equal ratio, fermented at 37°C up to 20 days. The activity was more predominant in panchagavya distillate of Gir and Sahiwal compared to Tharparkar and Karan Fries.

KEYWORDS: Antibacterial, antifungal activity, panchagavya, indigenous cow, distillation

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1. INTRODUCTION

In the Indian tradition it is believed that all the living beings in this world are made up of five elements of nature, i.e., Earth, Water, Air, Fire and Space, together known as panchabhootas. Any disturbance in these ratios of five elements can cause disease. On this principle, different remedial systems were developed and mentioned in ancient Ayurvedic texts. Ayurveda is, one of the ancient yet living health traditional forms of medical practice. Originated in India, Ayurveda is commonly referred to as 'science of life' because the Sanskrit meaning of *Ayu* is life and *Veda* is science or knowledge. Charaka Samhita, Sushruta Samhita (~400 BC–200 AD) and Ashtanga Hridaya of Vagbhata are main classics, which give detailed descriptions of over 700 herbs and 6,000 formulations (Patwardhan, 2014). One of these documented ayurvedic organic formulations is "panchagavya" that is having the blend of five cow-derived products i.e. milk, ghee, curd, dung and urine (in Sanskrit, all these products are individually called as "Gavya") (Parkavi et al., 2021) and it is being mentioned in Apasmar-Chikitsa- Adhyaya for treatment of Apas- mar (cognitive and memory decline), Kamala (Jaundice), and Jwara (fever). Limited and contradictory studies are available related to bioactive properties like hepatoprotective(Achliya et al., 2004, 2003) immunostimulant (Gajbhiye et al., 2014), anti-inflammatory (Dhama et al., 2005) antioxidant (Sharma, 2009) and antimicrobial activity (Ram et al., 2020: Joseph, and Sankarganesh, 2011; Mathivanan et al., 2006; Patel et al., 2018; Subramaniam, 2005) of panchagavya. Panchagavya is majorly studied for the application in veterinary sciences and agronomy (Chaudhari et al., 2018; Deepika et al., 2016; Jain et al., 2014; Kumar et al., 2015; Mathivanan et al., 2006; Mehala et al., 2021; Nagaraj and Sreenivasa, 2009; Natarajan, 2008; Paliwal et al., 2013; Tharmaraj et al., 2011; Yadav and Lourdraj, 2006) and recently in the area of nanotechnology (Arumugam et al., 2019; Tidke et al., 2019). However, most of the reported studies were majorly consist of preliminary data and devoid of indepth scientific evidence on method of preparation of panchagavya formulation with respect to its bioactive properties and characterization of the key components responsible for these properties. Thus, knowledge about the Panchagavya is very important for an agricultural based rural economy and has the potential of improving the financial condition of the farmers (Alves, 2009; Dhama et al., 2013). This formulation is of excellent therapeutic potential that is of great scientific and medical interest and could be used as an efficient alternative to the fight toward antibiotics resistance in the microbial pathogen (dos Santos et al., 2015).

No systematic data was available for the effect of fermentation temperature and source of basic five ingredients. The aim of this work was to standardize the method for preparation of the panchagavya formulations with antimicrobial potential, by varying the ratio of five basic ingredients, time and temperature of fermentation and comparing the antimicrobial potential of different indigenous and cross bred cow's panchagavya prepared using standardized conditions.In this work, we described the simplified yet scientific approach for standardization of method for the preparation of panchagavya formulations with high antifungal potential. Further, the effect of two specific physiological conditions that are heifer and lactation are also studied to compare the antifungal potential of panchagavya formulations in diverse indigenous as well as cross bred cattle.

2. MATERIALS AND METHODS

MRS (de Man, Rogosa, Sharpe) borth, BHI (Brain Heart Infusion) broth, Nutrient broth and Agar powder used in the study were purchased from HiMedia Pvt. Ltd. (Mumbai, India). Three Gram positive (*Bacillus cereus* NCDC-66, *Bacillus subtilis*NCDC-70 and *Staphylococcus aureus*NCDC-109) and two Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas fluorescens* NCDC-316) bacterial strains, and two molds (*Penicillium camemberti* NCDC-56and *Aspergillus niger* NCDC-55) and two yeasts (*Candida butyri* NCDC-280 and *Rhodotorula glutinis* NCDC-51) were resourced from the lab depository of National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute, Karnal. Unless noted otherwise, chemicals were of analytical grade.

2.1. Sample collection and preparation of basic ingredients

The urine and dung samples were collected from Live Stock Research Centre of ICAR-National Dairy Research Institute (NDRI), from respective cattle Sahiwal, Tharparkar, Gir and Karan Fries [*Bostaurus*, 105.5±2.8 kg body mass (mean±SD)] in the presence of veterinary doctor from heifer and lactating animals without any stress during sample collection. All the animals were clinically normal and divided into two groups; Heifer (age between 17^{th}-18^{th} months), and lactating (80^{th}-100^{th} days). The procedure for the urine samples treatment is same in all the conditions and describes briefly. Approx. 500 ml of second morning voids urine samples were collected by stimulating the animal through manual perineum massaging. The collected samples were immediately transferred to the lab and analysed for any debris to rule out any contaminants in the samples. Initially the samples were filtered by muslin cloth followed by centrifugation at 7000 rpm for 20 minutes to allow settlement of any cell debris and particulate matter. Raw milk from the different breeds; Sahiwal, Tharparkar, Gir and Karan Fries was also collected in the morning hours

from Livestock Research Centre, ICAR-National Dairy Research Institute. Curd was prepared using the culture NCDC 167 resourced from National Collection of Dairy Cultures (NCDC), National Dairy Research Institute, Karnal and ghee sample was prepared by direct creamery method (De, 2010).

2.2. Culture conditions

Potato dextrose broth medium and Agar powder used in the study were purchased from HiMedia Pvt. Ltd. (Mumbai, India). Two molds (*Penicillium camemberti* NCDC-56 and *Aspergillus niger* NCDC-55) and two yeasts (*Candida butyric* NCDC-280 and *Rhodotorula glutinis* NCDC-51) were resourced from the lab depository of National Collection of Dairy Cultures (NCDC), ICAR-National Dairy Research Institute, Karnal. Unless noted otherwise, chemicals were of analytical grade.

2.3. Standardization of method for panchagavya formulation preparation

In the process of standardization, panchagavya formulation was prepared using Sahiwal breed's five basic ingredients (Milk, Curd, Ghee, Urine and Dung). All the five basic ingredients were mixed in three different ratios with increasing proportion of urine in Ratio A (20%), Ratio B (50%) and Ratio C (70%) and labeled as RA, RB and RC, respectively. The Panchagavya formulation was prepared in three steps. In the first step, both the milk and ghee were heated to 40°C and mixed using the magnetic stirrer followed by blending using a hand blender for uniform mixing. In the second step, the curd sample and urine were also mixed in similar way. In the third step, dung filtrate was prepared by mixing dung and water in equal ratio followed by filtration with muslin cloth. All the three different mixtures obtained through step 1, 2 and 3 were mildly heated to 40°C and again mixed using hand blender. The prepared panchagavya formulations were then allowed to ferment at two different temperatures i.e. 25°C and 37°C up to 30 days and analyzed for antifungal activity at 10 days interval.

2.4. Measurement of pH

The pH of panchagavya and its distillates was determined prior to and during fermentation up to 30 days at 10 days interval. For pH measurement, digital pH meter (Eutech instruments, Singapore) was used.

2.5. Distillation of Panchagavya formulations

The panchagavya formulations in three different ratios (RA, RB and RC) kept for fermentation at two different temperatures (37 and 25°C). Prior to fermentation and after 10 days interval, up to 30 days 20 g panchagavya samples were distilled at 100°C till 15 ml (approximately) of distillate was collected. The distilled samples obtained from RA, RB and RC were labeled as DRA, DRB and DRC, respectively and used for analysis.

2.6. Screening of antimicrobial potential of panchagavya formulation

Antimicrobial activity was evaluated against three gram positive and two-gram negative bacterial strains, and two molds and two yeasts using agar well diffusion assay as per method of Schillinger and Lucke, 1989 with some modifications. The method is based on the principle that involves the ability of one microorganism to inhibit the growth of another, as exhibited by clear zone of inhibition. An aliquot of 10 g of panchagavya sample was added to 90 ml of distilled water (40°- 45°C). It was then mixed thoroughly, followed by primary filtration in a filter paper (Whatman no. 42) and secondary by syringe filter of pore size 0.45 µm. The sterile filtrate (raw preparations) RA, RB and RC and the distilled samples obtained from RA, RB and RC labeled as DRA, DRB and DRC, respectively analyzed for antifungal activity. The standardized conditions further used for the panchagavya preparation, from different indigenous and cross bred cows (both lactating and heifer) and analyzed for antimicrobial activity. In case of panchagavya prepared from the heifer, only the urine and dung were collected from heifer and the rest of the ingredients were obtained from lactating animal of their respective breed. To check the antimicrobial activity, nutrient agar plates (15–20 ml) were made and allowed to solidify. Then the nutrient agar plates were overlaid with 7 ml of soft agar (0.7% agar) inoculated with 100 µl of overnight active culture of indicator strains (Pathogen). The soft agar was allowed to solidify. The plates were refrigerated at 4°C for 1h before several wells were punched out of the agar with sterile glass borer. The wells were then filled with 100 µl of prepared samples. The plates were once again refrigerated at 4°C for 3–4 h to facilitate the diffusion of supernatant and were incubated at 37°C for 24–48 h. The diameter of zone of inhibition extending laterally around the well was measured and a clear zone of 1 mm or more was considered positive inhibition.

2.7. Statistics

Samples were taken in duplicates for all the parameters. Three trials were conducted for each experiment for consistency of the results. The data generated from various trials under experiments were pooled and analyzed by statistical method of ANOVA and Mean±S.E using PRISM software (Graph-Pad Prism Software, San Diego, CA). Duncan's multiple range tests and critical difference were determined at 5% significance level. Statistically analyzed data was tabulated and interpreted.

3. RESULTS AND DISCUSSION

3.1. Effect of fermentation time and temperature on pH profile

of panchagavya samples

With the proven and stabilized fact that the World is facing a rising problem of multidrug-resistant microorganisms, it is immediate requirement to determine the substitute to overcome the concern. Therefore, abundant experiments have been performed to identify novel compounds, especially natural origin are of extremely importance (Organization, 2014, dos Santos et al., 2015). A characteristic feature of *Staphylococcus aureus* is its ability to acquire resistance to antimicrobial agents. There is a need, therefore, for new approaches to combat this pathogen; for example, employing a combination of plant-derived products and antibiotics to overcome bacterial resistance. Indigofera suffruticosa is a plant popularly used to treat infections and has verified antimicrobial action. Here, we investigate the antimicrobial activity of different extracts from I. suffruticosa against *S. aureus* and their synergistic effects with erythromycin. Leaves of I. suffruticosa were extracted sequentially using diethyl ether, chloroform and acetone and the antimicrobial activity of each extract then tested against nine clinical isolates of *S. aureus*. Minimal inhibitory concentration (MIC. In this context, the identification and characterization of panchagavya formulation having antimicrobial activity (AMPs) will become to be substantial alternate and suitable option for the multidrug-resistant microorganisms. We prepared powerful formulation of panchagavya by mixing the five basic components viz.; cow milk, ghee, curd, urine and dung obtained from indigenous breed Sahiwal. The influence of temperature and time of fermentation on pH of panchagavya samples was assessed by potentiometery using the pH meter. The pH is an important factor that affects almost all the physical, chemical and biochemical reactions that occur during the process of fermentation. The effect of days of fermentation and basic ingredients ratio on pH profile of panchagavya fermented at 37°C is presented in Figure 1. Prior to fermentation, the pH value of raw preparations with Ratio A (RA), Ratio B (RB) and Ratio C (RC) was 7.49±0.09, 8.02±0.16 and 8.23±0.26 respectively while distillates of same samples; DRA, DRB and DRC increased their pH towards the alkaline side. However, fermentation at 37°C up to 30 days, shifted the pH value of RA towards acidic side as the days of fermentation progressed, while for RB and RC, remains in alkaline side as the urine content in the formulation was 50 and 70%, respectively. Further, no significant difference (p<0.05) on pH value was observed between distilled un- distilled panchagavya samples at respective day of fermentation. Similar trend was observed in the pH profile of panchagavya samples fermented at 25°C in all the three ratios; RA, AB and RC and their respective distillate.

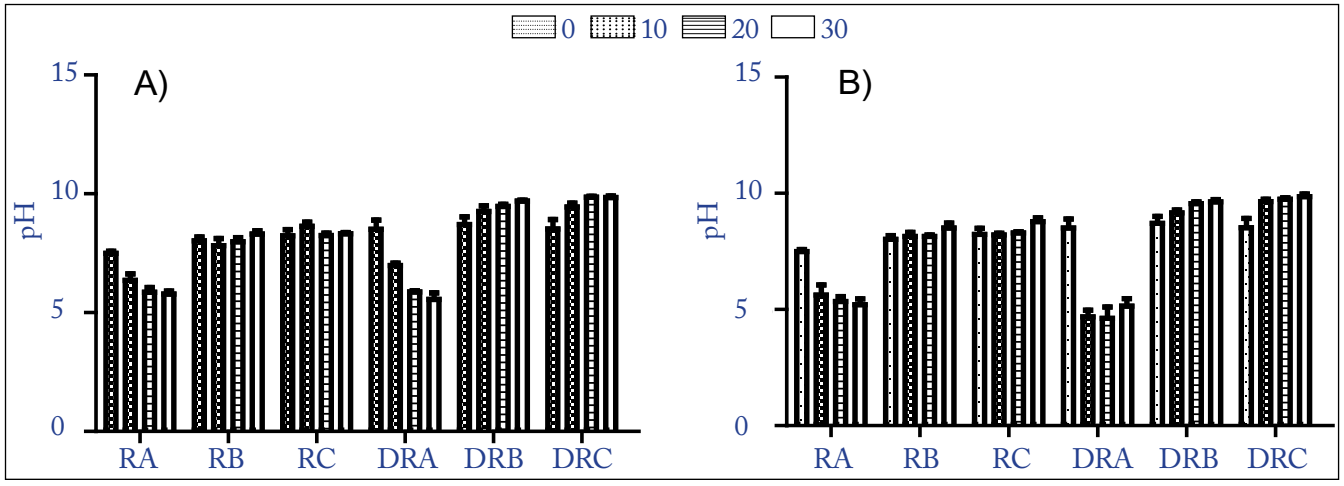

Figure 1: Effect of fermentation on pH profile of panchagavya samples fermented at A) 37 °C and B) 25°C

3.2. Effect of ingredients ratio on pH profile of panchagavya samples

In both the temperature, pH of RA and DRA is observed to be significantly different (p<0.05) from pH on 0th day with its pH on 10th day and no significant difference (p>0.05) was observed their after with increase in days of fermentation. In case of RB, RC and its distillate no significant difference (p>0.05) was observed in pH with the increase in days of fermentation except in DRC where significant difference (p<0.05) was observed between the pH on 0 day and 30th day at both temperatures. The difference observed in pH value was due to the change in the ratio of five basic components in panchagavya formulations. In RA, milk and curd were present in higher concentration which may lead to the production of lactic acid along with other organic acids with the help of lactic acid bacteria of curd. For ratio RB and RC, the pH values were towards alkaline side as the urine content in the formulation was 50% and 70%, respectively. Distillate of RA i.e. DRA showed pH value in acidic side while DRB and DRC showed pH

more towards alkaline side as compared to raw preparation of panchagavya formulations.Method for preparation of panchagavya formulation has been standardized with respect to ratio of five basic ingredients (Ratio A, B and C), time and temperature of fermentation on the basis of antimicrobial activity. The standardized conditions showing potent antimicrobial activity comprises of PG preparation as per Ratio A, fermented at 37°C for 20 days followed by distillation.

The influence of temperature and time of fermentation on pH of panchagavya samples was assessed by potentiometery using the pH meter. The difference observed in pH value may be due to the change in the ratio of five basic components in panchagavya formulations. In RA, milk and curd were present in higher concentration leading to the production of lactic acid along with other organic acids with the help of lactic acid bacteria of curd that converts lactose and other sugar into lactic acid (Widyastuti and Febrisiantosa, 2014). These finding pertains to the decrease in pH with increase in fermentation days of panchagavya as reported in literature (Mathivanan et al., 2006). For Ratio B and C, the pH values were towards alkaline side as the urine content in the formulation was 50 and 70%, respectively. Urea contributes majorly (65–90%) of the total nitrogen in cow urine and on hydrolysis it gets converted to ammonium nitrogen (Whitehead and Raistrick, 1993) which is responsible for alkaline pH of the formulation. Further distillation increased the concentration of ammonia and shifted the pH more towards alkaline side.

3.3. Effect of fermentation on antibacterial activity of panchagavya

The antimicrobial activity of the Panchagavya samples was studied against five indicator bacterial strains, three Gram positive (*Bacillus cereus* NCDC-66, *Bacillus subtilis* NCDC-70 and *Staphylococcus aureus* NCDC-109) and two Gram negative (*Escherichia coli* ATCC 25922 and *Pseudomonas fluorescens* NCDC-316) and four fungal strains, (*Penicillium camemberti* NCDC-56 and *Aspergillu sniger* NCDC-55, *Candida butyri* NCDC-280 and *Rhodotorula glutinis* NCDC-51). The zone of inhibition above 9 mm in diameter was taken as positive result. The panchagavya formulations and their respective distillate showed no antibacterial activity against gram negative bacteria in all the three ratios at both the temperature of fermentation up to 30 days. Prior to fermentation, similar results were observed against gram positive bacteria however as the fermentation progressed, DRA fermented at 37°C showed maximum antibacterial potential after 20 days of fermentation against two-gram positive bacteria viz; *Bacillus cereus* NCDC-66 and *Bacillus subtilis* NCDC-70 with highest zone of inhibition i.e., 16.75±2.25 and 15.75±0.75 mm, respectively. The results revealed that the antibacterial activities of panchagavya formulations prepared in different ratios were not consistent as compared to antifungal activity and the distillates were found to be more potent than the panchagavya formulations. From the results it was found that the Ratio A fermented at 37°C for 20 days showed the most potent activity. The possible hypothesis may be due to concentration of antimicrobial metabolites in the distillate where in it gets concentrated whereas in Panchagavya formulation in the sample preparations steps it gets diluted. No antimicrobial activity was observed on the 0 day (day of preparations) which was similar to the other reported literature (Mathivanan et al., 2006; Suresh et al., 2011).

3.4. Effect of fermentation on antifungal activity of panchagavya

Antifungal activity of panchagavya formulations and their respective distillates against selected indicator yeast and mold was evaluated. The results obtained in the present work revealed that antifungal activity of panchagavya formulation depends on ratio of five basic ingredients, time and temperature of fermentation and also distillation of samples affects the antifungal efficacy of panchagavya. At 0 day (day of preparation) the panchagavya formulations (raw preparations) along with the distillates did not show any antifungal activity against the selected yeasts and molds. After 10 days of fermentation, among the raw preparations, RB and RC (fermented at both 37°C and 25°C) showed antifungal activity against yeast (*C. butyri* and *R. glutinis*) and mold (*P. camemberti*). However, only RC (fermented at both 37°C and 25°C) showed activity against *As. niger*. Further, on distillation of same samples, increase in antifungal efficacy was observed. The distillate obtained from sample RA i.e., DRA (fermented at both 37°C and 25°C) showed maximum and consistent zone of inhibition against all the selected fungi (Figure 2 and 3). The maximum zone of inhibition was observed against *P. camemberti* (29.33±3.18) and minimum against *As. niger* (21.5±5.35). Distillates of ratio B was showing antifungal activity against all the selected indicator at 37°C but no zone of inhibition was observed for the same sample fermented at 25°C (except against *As. niger*) which shows that as the temperature of fermentation increases the type of metabolites in the panchagavya formulation also varies. After 20 days, DRA (fermented at both 37°C and 25°C) showed consistent and potent antifungal activity, but the zone of inhibition was more as compared to that observed after 10 days of fermentation against all the selected indicator fungi. Distillate DRB and DRC (fermented at both 37°C) did not show any activity while DRB (fermented at 25°C) showed 16.5±0.5 and 10.5±0.5 mm zone of inhibition against *C. butyri* and *As. niger*, respectively. DRC (fermented at 25°C) showed 12.5±2.5 mm zone of inhibition against *As. niger*. Increase

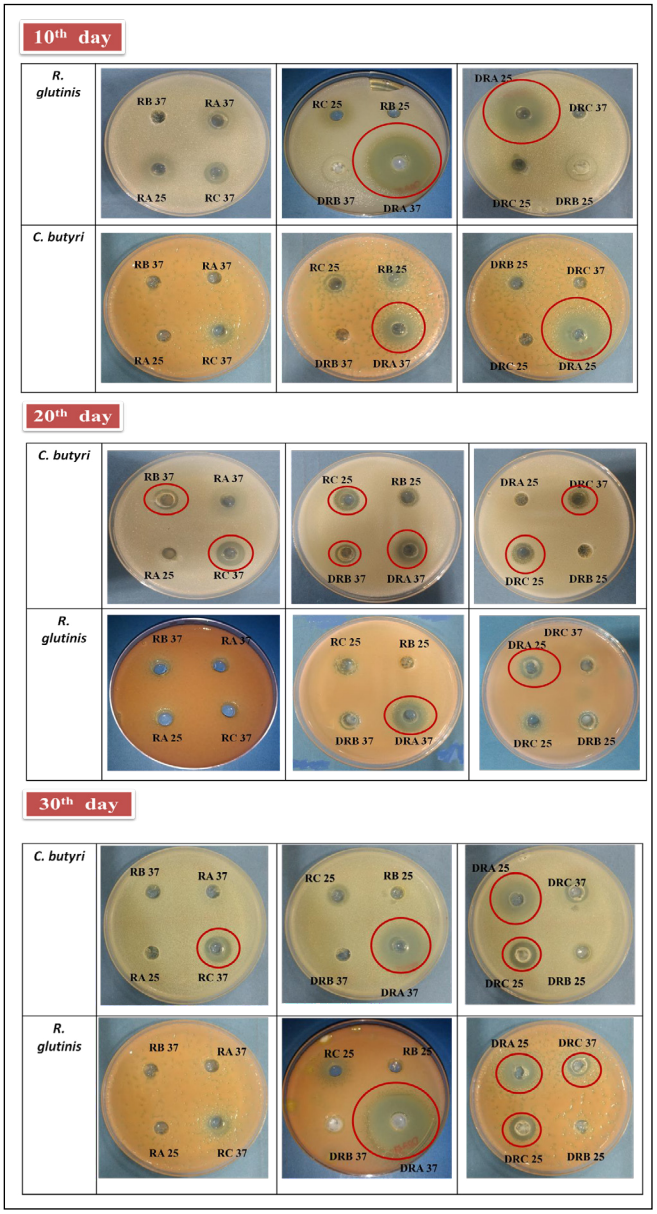

Figure 2: Effect of fermentation on antibacterial activity of panchagavya against yeast

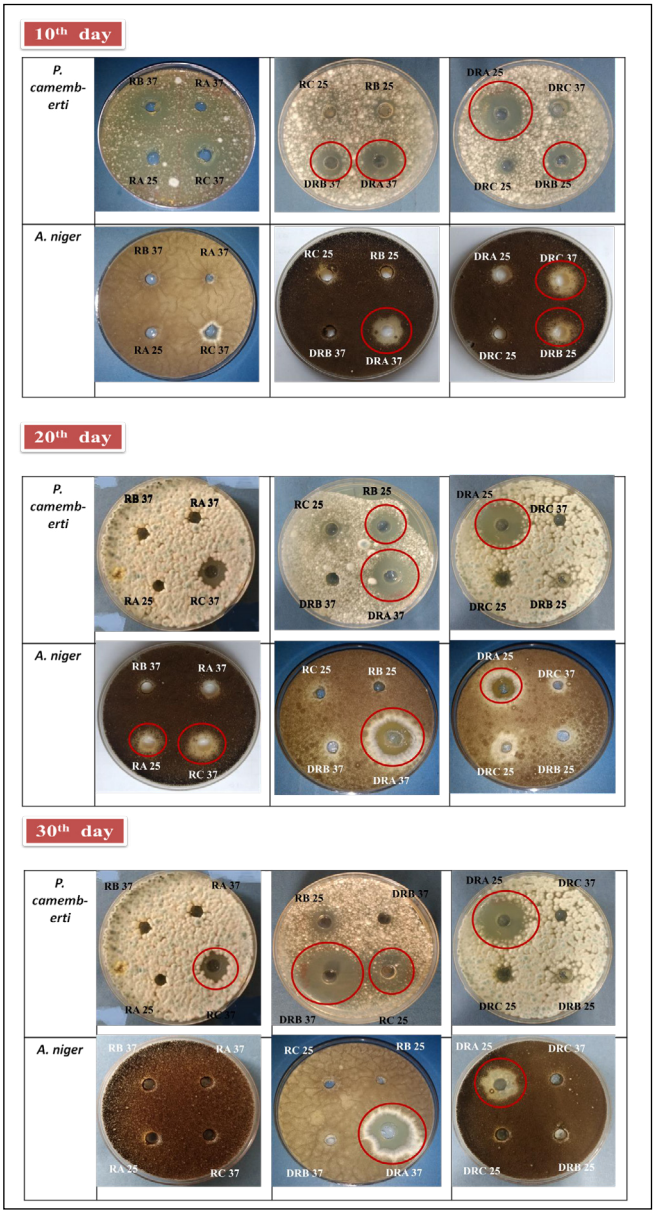

Figure 3: Effect of fermentation on antibacterial activity of panchagavya against mold

in time of fermentation up to 30 days showed no significant difference in antifungal activity of raw preparations viz. RB and RC as well as distilled samples DRB and DRC at both the temperature of incubation.

Among all the raw preparations and distillates of panchagavya formulations, DRA fermented at 37°C showed potent antifungal activity with zone of inhibition in the range of 22 to 28.83 mm. After the distillation of PG, antimicrobial activity increased which might be due to the process of distillation as the compounds/metabolites having the antimicrobial property formed during fermentation got concentrated in the distillate. Ratio of five basic ingredients also influenced the antifungal activity, especially proportion of urine in formulation. It was observed that in the distillates of panchagavya formulations antifungal activity decreased as the proportion of urine increased. Ratio A had minimum percentage of urine i.e., 20%. It is evident from the data that distillate of ratio A (DRA) had maximum antifungal activity. Effect of temperature of fermentation on antifungal activity was also observed, as in the distillate DRA which was obtained from panchagavya formulation RA fermented at 37°C the zone of inhibition was maximum than one fermented at 25°C.

Joseph and Sankarganesh, 2011, reported the antifungal efficacy of panchagavya following the dilution method, in which different dilutions of Panchagavya were prepared

using distilled water containing water agar medium. They observed that the growth ratio of fungal colonies decreased with decreasing dilution of panchagavya in medium. Different analysis and the more fungi specific approach used in the present study also showed the similar results. No activity on 0 day in both raw preparations and the distillate indicates that the fermentation plays an important role in the possible antimicrobial metabolites formation. The Lactobacillus *spp.* that are abundant in the curd are also reported to produce the broad spectrum of antimicrobial substances, organic acids produced by these lactic acid bacteria also affect the growth of pathogen and could be toxic to the microbes (Alvarez-Olmos and Oberhelman, 2001). The unique micro flora of cow dung which has abundant number of lactobacilli, bacilli and cocci and also antifungal substances which inhibits growth of Corprophilus fungi (Lehr et al., 2005) and patulodin like compound in cow dung having antifungal activity (Dorothy and Frisvad, 2002) and creatinine, uric acid and phenolic acids present in the cow urine might play a possible role in the antimicrobial activity of the fermented panchagavya formulations as evident from the present study. Hence, the conditions for the preparation of panchagavya formulation having potent antifungal activity against fungal cultures was standardized, which included the preparation of panchagavya as per ratio A, fermentation at 37°C for 20 days followed by its distillation.

3.5. Antifungal efficacy and pH profile of panchagavya formulations prepared from indigenous and cross bred cows under standardized conditions

The standardized method comprises of basic ingredients in Ratio A, fermentation temperature 37°C for 20 days followed by distillation was used to prepare panchagavya formulations from different lactating and heifer indigenous (Gir, Tharpakar and Sahiwal) and cross bred (Karan Fries) cows as mentioned in Table 1. Comparison of pH revealed that panchagavya formulations and their distillate prepared under standardized conditions from the indigenous breeds and cross bred cows (both lactating and heifer) were in the acidic side after the 20 days of fermentation at 37°C. The possible reason may be the presence of Lactobacillus spp. that converts lactose and other sugar into lactic acid. (Widyastuti and Febrisiantosa, 2014) reported that the well-known characteristics of these types of lactic acid bacteria were to produce acid that results in decrease in pH. The finding in the present work pertains to the decrease in pH with increase in fermentation day of panchagavya as reported by (Mathivanan et al., 2006).

Table 1: Antifungal activity of panchagavya formulations prepared from different indigenous and cross-bred cows

Distilled PG	Yeast	Mold		
	Zone of inhibition (mm)			
	C. butyri	*R. glutinis*	*P. camemberti*	*As. niger*
Gir	21.00±1.52^{B}	21.67±2.33^{B}	24.33±1.20^{C}	18.33±1.20^{BC}
Gir's Heifer	21.50±0.50^{B}	29.00±1.00^{A}	30.00±1.00^{A}	23.50±0.50^{A}
Tharparkar	17.66±1.20^{C}	20.66±1.76^{B}	27.33±0.67^{B}	20.33±1.86^{B}
Tharparkar's Heifer	16.50±0.50^{C}	16.50±1.50^{B}	20.00±1.00^{D}	15.50±0.50^{C}
Sahiwal	26.16±0.72^{A}	21.50±2.17^{B}	26.17±1.02^{BC}	21.00±0.57^{AB}
Sahiwal's Heifer	23.50±1.50^{AB}	27.50±1.50^{A}	27.00±1.00^{BC}	20.00±1.00^{B}
Karan Fries	23.66±1.85^{AB}	19.66±1.76^{B}	21.33±1.45^{D}	16.33±0.88^{C}
Karan Fries's Heifer	16.50±0.50^{C}	21.00±2.00^{B}	27.00±1.34^{BC}	21.00±1.00^{AB}

Values are presented as Mean±SE, n=3. ABCD- Mean with different superscripts within columns differ significantly (p<0.05); # zone of inhibition including well diameter (9 mm)

The raw preparations of the panchagavya formulations prepared under standardized conditions from different indigenous and cross bred cows (both lactating and heifer) did not show any antifungal activity. However, in case of their respective distillates, the antifungal activity of panchagavya samples showed significantly higher zone of inhibition in the panchagavya prepared using dung and urine of heifer as compared to the lactating animals (Table 1). Among different breeds, panchagavya samples of Gir and Sahiwal cow showed better antifungal activity followed by Tharparkar and Karan Fries cows. Specifically, mentioning the fold change difference in Gir Hiefer v/s Gir Lactation showed more than1.2-fold increment in the effect of Gir Lactation formulation for all the fungus tested.

4. CONCLUSION

Panchagavya formulation is less explored especially from bioactive property point of view. The distillate obtained

from fermented panchagavya formulation RA (DRA) showed potent antimicrobial activity and the highest zone of inhibition was observed on 20th day of fermentation at 37°C. The parameters such as individual ingredients ratio, time and temperature of fermentation and breed of cow significantly affect the bioactive property. Panchagavya formulation prepared using heifer's urine and dung from indigenous breed Sahiwal and Gir showed most potent antifungal activity.

5. FURTHER RESEARCH

Because of its potent antifungal activity, panchagavya has wider scope in organic farming as a bio pesticide. Since panchagavya is made of all organic ingredients, it doesn't pose any threat to environmental pollution or harm to plants. Application of panchagavya in agriculture sector is a way to increase the farmer's income, reduce pesticide residues in foods and also helps to maintain consumer health. Further, the characterization for key bioactive compounds using high end techniques viz., UV-VIS spectra, attenuated total reflectance- Fourier Transform Infrared (ATR-FTIR) Spectroscopy, high-resolution LC-MS/MS-based identification will be helpful in the determination of potential bioactive peptides in the panchagavya formulation.

6. ACKNOWLEDGEMENT

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