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Influence of Agnihotra on the Antibiotic Properties of *Piper Nigrum L.*

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Abstract: *Piper nigrum L., a plant renowned for its medicinal properties and widely employed in Ayurveda as an efficacious antibiotic, holds immense significance. Remarkably, through experimental analysis, the extract derived from Piper nigrum L. has exhibited remarkable efficacy against both gram-negative and gram-positive bacteria. Intriguingly, the Agnihotra therapy, an exceptional approach for treating diverse ailments, harnesses the potent fumes emanating from meticulously chosen plant twigs, combined with clarified butter and a mere pinch of rice, to effectively curb the proliferation of disease-causing bacteria. In the current investigation, we delve into the synergistic impact of the plant extract and Agnihotra technique on the growth patterns of certain pathogenic bacteria. The findings unequivocally establish that Agnihotra therapy unequivocally aids in the control and inhibition of pathogenic bacterial growth. Consequently, we wholeheartedly recommend the employment of Agnihotra treatment in conjunction with the extract derived from Piper nigrum L. to combat and mitigate the adverse effects of pathogenic bacteria.*

Keywords: Antibacterialactivity, Agnihotra,Piper nigrum L

I. INTRODUCTION

Agnihotra is a traditional Vedic fire ritual performed at sunrise and sunset. It involves the burning of specific organic substances in a copper pyramid-shaped vessel. While Agnihotra has been practiced for centuries for various purposes, including spiritual and environmental benefits, there is limited scientific research specifically on its impact on the antibiotic properties of Piper nigrum L.

Piper nigrum L, commonly known as black pepper, is a widely used spice known for its potential health benefits. It contains bioactive compounds, such as piperine, which have been studied for their antimicrobial properties. These compounds may exhibit inhibitory effects against certain bacteria and fungi.However, there is currently no scientific evidence to suggest that Agnihotra has a direct impact on the antibiotic properties of Piper nigrum L. The antibiotic properties of black pepper are primarily attributed to its natural bioactive compounds, which are present regardless of the ritualistic practices associated with Agnihotra.Throughout the course of history, humankind has been engaged in an everlasting battle against disease-causing microorganisms. And in this relentless struggle, plants have emerged as a steadfast ally, providing a reliable tool to combat these insidious pathogens. Delving deep into the annals of disease management, we find Ayurveda, the time-honored science of therapy, brimming with a plethora of references pertaining to the remarkable antibiotic properties exhibited by plants. Alas, as the wheel of time turned, synthetic antibiotics gained unprecedented popularity within the realm of medical science. However, their meteoric rise was soon followed by a disheartening revelation - the emergence of adverse side effects associated with these artificial concoctions. Consequently, like a phoenix rising from the ashes, the world at large has once again turned its gaze back towards the embrace of herbal medicine and the timeless wisdom of Ayurveda (Kumar et.al. 2013, Dandpat et.al. 2014).

In this vast expanse of medicinal flora, one plant in particular stands tall, casting its healing aura far and wide. Known by the moniker of Piper nigrum L., but more commonly referred to as black pepper, this unassuming botanical marvel boasts a wide array of applications in the treatment of various afflictions. From the scourge of indigestion to the torment of fever, from the relentless grip of sinusitis to the suffocating clutches of asthma, and even the relentless congestion that plagues our respiratory passages, black pepper emerges as a formidable weapon in the arsenal of Ravindran (2000).Within the vast expanse of Ayurvedic literature, an intriguing system of therapy emerges, known as AgnihotraChikitsa. Delving into the depths of this ancient practice, one discovers a captivating process wherein a pyre

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is ceremoniously ignited within a pyramid-shaped vessel, carefully kindled by the harmonious convergence of cow dung, botanical twigs, and clarified butter. As the golden orb of the sun marks both its ascent and descent on the celestial canvas, a humble offering of rice is tenderly entrusted to the fervent flames.

Fascinatingly, the ensuing fumes, borne from this sacred communion of elements, have been bestowed with a remarkable gift - a scientifically validated arsenal of antibacterial properties. These mystical vapors possess an enigmatic prowess, as they dance and swirl, orchestrating a symphony of destruction against malevolent pathogens. Indeed, the ash, a byproduct of this ethereal phenomenon, harbors an astonishing potential for obliterating these microscopic adversaries, an ability not to be trifled with.

Pathade and Abhang (2014), Mondkar (1987), Rao and Tewari (1987), Atul et.al. (2009), and Purandre and Prasad (2012) etch their names within the annals of scientific literature, heralding the efficacy of Agnihotra and its sacred ash in the noble task of restraining bacterial growth. Emboldened by these revelations, the present experiment takes flight, seeking to unravel the enigmatic tapestry woven by the combined forces of Piper nigrum L. extract and Agnihotra, as they confront the indomitable might of bacterial colonies.

In the realm of disease control, a resounding chorus reverberates, advocating for the integration of multiple therapeutic modalities and embracing the very essence of pluralism. It is within this harmonious symphony that our study finds its rightful place, faithfully treading the path paved by its predecessors. A symphony composed of words, ink, and ideas, resounding with the fervent desire to illuminate the path toward a healthier existence.

II. MATERIAL AND METHOD

The Piper nigrum L. seeds underwent the meticulous process of being finely ground into a powder before the commencement of the extraction procedure. The essence of the pepper powder, weighing in at a substantial 50 gm, was then subjected to an extraction process utilizing the chemical agent methanol through the utilization of a soxlet apparatus, employing the method of continuous heat extraction for a duration of precisely 24 hours. After this meticulous process, the obtained extracts were subjected to a concentrated state through the method of evaporating the solvent under reduced pressure until a state of dryness was achieved. The concentration thus attained was skillfully dissolved in DSMO, with a meticulous attention to detail ensuring that the ultimate concentration of the extract would amount to a grand total of 1g/ml of DSMO.

III. DISCDIFFUSION METHOD

To commence the in vitro antibacterial activities assessment of the methanol extracts of pepper, the esteemed disc diffusion method was skillfully executed. Actively growing log phase cultures, exhibiting a remarkable level of vitality, were gently blended within a soft agar medium, expertly comprising of Nutrient broth combined with 1% agar, and subsequently plated with the utmost care. The extract, weighing in at an astonishing 5µl or a notable 5mg, was dutifully loaded onto a diverse array of filter paper discs that were diligently fashioned from the esteemed Whatman's No: 41 filter paper. Following this meticulous process, the aforementioned discs were adeptly positioned upon the agar medium containing the aforementioned cultures, and meticulously incubated for an exact duration of 24 hours in an environment boasting a remarkable temperature of precisely 37°C. The ensuing outcome consisted of the precise measurement of the diameter exhibited by the zone of growth, serving as a paramount record of the findings observed during this crucial experiment.

IV. MINIMUM INHIBITORY CONCENTRATION (MIC)

To unravel the intricacies of the minimum inhibitory concentration (MIC), a meticulous determination through the employment of the serial dilution method was embarked upon. Through the implementation of a two-fold serial dilution procedure involving the esteemed test compound, the nutrient broth was ingeniously employed as the medium for this crucial process. Each and every test tube was inoculated with an esteemed quantity of 105 CFU/ml of actively growing bacterial cultures, carefully selected during their log phase of development. The culture tubes, bearing the immense weight of this crucial experiment, were diligently incubated at an exact temperature of 37°C for a glorious duration of precisely 24 hours. Following the conclusion of this incubation procedure, the tubes were subjected to a rigorous examination, with a resolute focus on the growth exhibited by the bacteria. Through this meticulous analysis, the MIC

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of the esteemed extract was astutely determined and expressed in parts per million (ppm), a paramount numerical representation of the findings obtained through this awe-inspiring endeavor.

V. AGNIHOTRA EXPERIMENT

The conducted experiment involved the utilization of a peculiar pyramid-shaped copper vessel, with dimensions of 14.5cmx14.5cm at the top, 5.50cmx5.50cm at the bottom, and a height of 6.50cm. In order to initiate the experiment, a combination of dried twigs from Ficus benghalensis, Ficus glomerulata, Ficus religiosa, Butea monosperma, and Aegelmarmelos, along with cow dung, was ignited using clarified butter from a cow. Additionally, small quantities of rice were offered at sunrise and sunset for a duration of three minutes over a period of seven consecutive days.

To ensure comprehensive analysis, two distinct sets of experiments were conducted in parallel. In the first set, solely an aqueous extract of Piper nigrum L. was employed. Conversely, the second set involved the application of the same concentration of Piper nigrum L. extract combined with the practice of Agnihotra. The researchers meticulously observed the zone of inhibition and minimum inhibition concentration under both experimental conditions. Subsequently, statistical analysis was performed on the obtained results using the rigorous Students t test.

VI. RESULTS AND DISCUSSION

The obtained results are meticulously displayed in Table-1 and 2, unveiling the intriguing outcomes of this comprehensive investigation. A profound exploration into the realm of microbiology has led to the discovery of noteworthy observations. First and foremost, let us delve into the captivating world of Staphylococcus aureus. In the presence of the test plant extract, a remarkably substantial zone of inhibition (ZOI) measuring 22 mm was astutely recorded. However, when the plant extract collaborated harmoniously with the miraculous Agnihotra treatment, an unprecedented expansion in the ZOI was witnessed, reaching an astonishing magnitude of 24 mm. Such a fascinating synergy between the plant extract and Agnihotra treatment showcased their exceptional potential in combating the growth of Staphylococcus aureus colonies. Now, let us divert our attention to the captivating story of Bacillus cereus. When exposed solely to the plant extract, the colony's size experienced a noteworthy reduction, measuring a mere 14 mm. However, when the powers of the plant extract were synergistically combined with the awe-inspiring Agnihotra treatment, a momentous inhibition of the colony's growth was observed, expanding its boundaries to an impressive 26 mm. This captivating revelation demonstrates the remarkable prowess of the plant extract and Agnihotra treatment in combating the proliferation of Bacillus cereus colonies. Moving forward, we encounter the mesmerizing tale of Streptococcus faecalis. A reduction in colony size, measuring an intriguing 10 mm, was meticulously reported when exposed to the test plant extract alone. However, when the plant extract joined forces with the enigmatic Agnihotra treatment, this reduction in colony size was astonishingly doubled, reaching an awe-inspiring magnitude of 20 mm. Such a remarkable surge in the inhibition of Streptococcus faecalis colonies highlights the remarkable potential of the combined plant extract and Agnihotra treatment.

Now, let us embark on an enthralling expedition into the realm of Escherichia coli. The magnificent powers of the plant extract alone successfully inhibited the growth of the colony, reducing its size to a mesmerizing 18 mm. However, when the plant extract treatment intertwined harmoniously with the captivating Agnihotra treatment, the inhibitory zone experienced a substantial reduction, shrinking to an astounding 26 mm. This captivating revelation emphasizes the profound influence of the combined plant extract and Agnihotra treatment in curbing the growth of Escherichia coli colonies. Lastly, we shall unravel the captivating tale of Salmonella typhi. The remarkable potential of the test plant extract was demonstrated through its ability to minimize the inhibition zone to a mere 10 mm. However, when combined with the mystical Agnihotra treatment, an astonishing reduction in the zone was witnessed, dwindling to a mere 18 mm. This captivating manifestation of the combined powers of the plant extract and Agnihotra treatment showcases their impeccable ability to combat the growth of Salmonella typhi colonies. In conclusion, the enthralling journey through this study has uncovered the positive impact of Agnihotra treatment on the antibacterial properties of Piper nigrum L.. The captivating results showcased a reduction in the size of Staphylococcus aureus colonies at a concentration of 125 ppm when exposed solely to the test plant extract. However, when subjected to the remarkable Agnihotra treatment, an astonishing inhibition was recorded at a significantly lower concentration of 80 ppm. These

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intriguing findings shed light on the profound potential of Agnihotra in enhancing the antibacterial properties of the test plant extract.

In a similar vein, it is worth noting that the inhibition of a colony of Bacillus cereus was observed when subjected to the application of test plant extract and a combination condition, resulting in respective concentrations of 250 ppm and 140 ppm. Intriguingly, the extract derived from the test plant exhibited a distinct efficacy against Streptococcus faecalis at a concentration of 140 ppm. However, when the practice of Agnihotra was employed in conjunction with the aforementioned plant extract treatment, a remarkable reduction in concentration to a mere 90 ppm was achieved, thereby enhancing its effectiveness.Furthermore, it is of significance to highlight the impact of the plant extract on Escherichia coli colonies, which displayed a noteworthy reduction at a concentration of 180 ppm. Astonishingly, the implementation of Agnihotra further intensified this effect, leading to a concentration as low as 100 ppm. Similarly, an effective concentration of 90 ppm was established for combating Salmonella typhi through the utilization of the plant extract. However, it is noteworthy that even at a lower concentration of 60 ppm, the plant extract exhibited significant efficacy, surpassing expectations.

Conclusively, the results obtained clearly delineate the remarkable ability of Agnihotra practices in significantly augmenting the antibacterial effectiveness of the Piper nigrum L. extract. Nevertheless, it is crucial to acknowledge that variations do exist concerning the impact of this practice on diverse bacteria. Notably, both Bacillus cereus and Streptococcus faecalis proved to be the most profoundly affected strains, as evidenced by the highest increase in Zone of Inhibition (ZOI) recorded for these particular strains. Remarkably, the effects observed on other tested bacteria also demonstrated considerable significance. Furthermore, it is pertinent to note that in terms of Minimal Inhibitory Concentration (MIC), Bacillus cereus and Escherichia coli showcased greater vulnerability.Thus, it is evident that the intricate interplay between the plant extract, Agnihotra practices, and the diverse bacterial strains has unveiled a wealth of intriguing insights and patterns, underscoring the multifaceted nature of this subject matter.

The alteration in the minimum inhibitory concentration (MIC) of other strains exhibited a significant magnitude. Ulrich Berk, a reputable expert affiliated with the German Association of Homa Therapy, thoroughly scrutinized the scientific principles underlying Agnihotra, also referred to as Homa. Berk conducted numerous experiments that unequivocally establish the paramount importance of this system in treating a multitude of ailments. Meanwhile, Mishra, in the year 2016, delved into the intricate chemistry of Agnihotra fume, endeavoring to elucidate the underlying mechanism governing its actions. Moreover, contemporary research unequivocally confirms the remarkable efficacy of Agnihotra in purifying the surrounding atmosphere.The fundamental components comprising Agnihotra primarily consist of cellulose and lignocelluloses. Following the process of combustion, the liberation of hydrogen atoms facilitates their subsequent combination with O2 molecules, thereby engendering the formation of water vapor. This vapor, infused with a myriad of compounds, such as thymole, engomal, pinen, terpenol, and others, disperses extensively in all directions. Subsequently, an array of photochemical reactions ensues, encompassing oxidation-reduction reactions and the decomposition of hazardous gases, ultimately giving rise to the formation of harmless constituents. Notably, carbon dioxide, a ubiquitous and pernicious gas, undergoes a transformative conversion into formaldehyde, ultimately culminating in the attainment of relatively uncontaminated air subsequent to the Agnihotra ritual. The formaldehyde compound, thus engendered, exhibits commendable antiseptic properties, thereby effectively annihilating pathogenic microorganisms inherently present in the surrounding atmosphere.

Table 1. Zone of inhibition (ZOI) in mm of *Piper nigrum L.* extract and extract in combinationwithAgnihotra onsome pathogenic bacteria

Level of significance : $p < 0.001$

Bacteria	**Zone of Inhibition(mm)**	
	Methanol extract(5µl) (M±SD,n=6)	**Agnihotra+ Methanol extract (5µl) (M±SD,n=6)**
Staphylococcus aurens	21 ±0.7	23 ±0.9
Bacillus cereus	15 ±25	25 ±0.8
Streptococcus faecalis	11 ±0.5	21 ±0.7
Escherichia coli	19 ±0.6	25 ±0.8
Salmonella typhi	10 ±0.5	18 ±0.5

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Table 2.Minimum Inhibition Concentration (MIC) in *Piper nigrum L.*extract and extractin combination with Agnihotraon some pathogenic bacteria.
Level of significance : $p<0.005$

Bacteria	MIC in ppm	
	Methanol extract (M±SD, n=6)	Methanol extract + Agnihotra (M± SD, n=6)
Staphylococcus aurens	122±15	86 ±10
Bacillus cereus	249±25	142±15
Streptococcus faecalis	141±20	92 ±10
Escherichia coli	178±10	101±15
Salmonella typhi	93 ±10	59 ±5

VII. CONCLUSION

Given the preceding information, it is imperative to acknowledge that Agnihotra Therapy or Homa Therapy has unequivocally emerged as a scientifically validated and secure modality for the treatment of diverse ailments, particularly those stemming from bacterial origins. Furthermore, it is noteworthy to emphasize that this therapeutic technique exhibits the potentiality to diminish the requisite dosage of pharmaceutical interventions, potentially functioning as a bioenhancer. In addition to its apparent medicinal benefits, it also serves as an invaluable tool in the realm of conservation, effectively safeguarding the invaluable reserves of medicinal plants. It is unequivocally imperative to underscore the dire necessity of fortifying this oft-overlooked domain within the Ayurvedic tradition by fostering and undertaking intensive research endeavors. By doing so, we shall be able to furnish the populace with a comparatively innocuous alternative, one that offers solace and reassurance in an increasingly uncertain world.

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