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A Research Work on: Comparative Antimicrobial Study of Marigold (Tagetes Erecta L.) And Neem (Azadirachta Indica) Against Gram Positive S. Aureus Bacteria

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Abstract:
The most common cause of pyogenic infection of the skin and soft tissues in children is Staphylococcus aureus, a fast-emerging problem due to its accompanying significant cost and morbidity. The popularity of herbal medications has increased due to the search for cheaper and more accessible alternatives. However, data is still lacking to back up these claims. In this research work the main aim to increase the activity of neem against bacteria by incorporating flower extraction of African marigold. As both leave extraction of neem and flower extraction of marigold has potent inhibition properties against Staphylococcus aureus, a gram positive bacteria, so dual action can be achieved and the inhibition properties also get stimulated. Here the methanolic exaction of both plants were taken in an identical manner and incorporate in a culture of Staphylococcus aureus and check the zone of inhibition for neem extraction as well as neem and marigold combination extraction. The result of this combination shows dramatically increase in prohibition as compare to single neem extraction. So, the conclusion is that the combination therapy of Tagetes erecta L. and Azadirachta indica can increase the property of prohibition against gram positive bacteria.

Keyword: Azadirachta indica, Antimicrobial activity, Tagetes erecta L., Staphylococcus aureus.

Introduction:

Tagetes erecta L.:

It is a highly rich botanical diversity that nature has given upon us, with many different kinds of plants growing wild throughout our nation. Throughout India, the practice of using various portions of various medicinal plants to treat particular illnesses has been popular since antiquity (Bhattacharjee, 1998). The species Tagetes minuta, erecta, patula, and tenuifolia are the most widely distributed in the globe, particularly for ornamental uses. Native to southwestern North America, tropical America, and South America, the marigold (genus Tagetes) is a genus of over fifty species of annual herbs in the aster family (Asteraceae)(1,2). Marigold is an essential bedding plant and loose flower crop. It is much sought after for wedding decorations, festivals, rituals, and everyday worship. It yields flowers in a variety of lovely hues, shapes, sizes, and qualities. Many farmers are growing French marigold (T. patula) and African

marigold (T. erecta) commercially since they are easy to grow. In addition to unrelated plants from several families, the term "marigold" also refers to the pot marigold (genus Calendula).

Botanical Description:

Pusa Narangi Gainda: The plants are 60–70 cm tall on average. The double-type flowers have an orange hue and a diameter of 6-7 cm. Flowers in plains take 125–135 days to open. 250–300 q/ha is the possible yield of fresh flowers. Pusa Basanti Gainda are 60–70 cm tall on average. The flowers are double-type, 6-7 cm in diameter, and sulfur yellow in color. Flowers in plains take 135–145 days to open. 200–250 q/ha is the possible yield of fresh flowers.

Pusa Arpita: This deep orange cultivar is good to plant in plains and low hills during the wet season. The cultivar blooms between January and December. Desi gutta: Seasonal variations in plant height (40–50 cm in winter, 50–80 cm in rainy seas) it bears fruit abundantly. The dark crimson flower has 3–4 cm diameter, silky, shiny petals.

Active Ingredients:

Numerous chemical components have been extracted and their structures clarified from Tagetes species.They fall into the categories of carotenoids, flavonoids, phenolic compounds, thiophenes, and essential oils. Sterols, glycosides, gums, and mucilages were found in the crude successive extracts of Tagetes erecta roots after a preliminary phytochemical screening(2,3).

Essential Oil from Tagetes erecta

Tagetes erecta flowers were found to include d-limonene, ocimene, 1-linalyl acetate, 1-linalool, tagetone, and n-nonyl aldehyde in their essential oil. Subsequent investigations found that the essential oil of Tagetes erecta contained aromadendrene, phenylethyl alcohol, salicylaldehyde, phenylacetaldehyde, 2-hexen-1-al, eudesmol , tagetone, ocimene, linalyl acetate, and an unidentified carbonyl compound. The essential oil of Tagetes erecta flowers was found to contain three acyclic monoterpene ketones, namely 3,7-dimethyloct-1-en-6-one, 3,7-dimethyl-5-hydroxyoct-1-en-6-one and 3,7-dimethyloct-1,7-dien-6-one, along with myrecene, caryophyllene, p-cymene, d-carvone, and eugenol(3).

Flavonoids from Tagetes erecta

6-hydroxykaempferol-7-O-b-D-alloside was found in Tagetes erecta flowers. Quercetagetin, Quercetagetrin and 6-hydroxykaempferol-7-O-glucoside were among the other flavonoids found in the aqueous methanolic extract of the defatted flower heads of Tagetes erecta.

Carotenoids from Tagetes erecta

Epoxides such lutein 5, 6-epoxide and other lutein oxidation products were first found in the extracts of Tagetes erecta.

Phenolic Compound from Tagetes erecta

In addition to ethyl gallate and methyl-3,5-dihydroxy-4-methoxy benzoate, the dried flowers of Tagetes erecta were found to contain phenolic chemicals, including syringic acid(4).

Neem:

It is a member of the Meliaceae family of plants and is one of the most adaptable medicinal plants that has garnered notoriety throughout the world for its insecticidal and therapeutic qualities. Numerous research have demonstrated Azadirachta indica's benefits in both clinical and experimental contexts. Azadirachtin, an active ingredient taken from the Azadirachta indica tree, is one of the most promising natural compounds among natural goods. Its antiviral, For a number of years, antifungal and antibacterial have been recognized. The phrase "nimbati swasthyamdadati," which translates to "to give good health," is the source of the Sanskrit name "nimba." The advantages of neem are mentioned in the historic texts "Charak-Samhita" and "Susruta-Samhita," which serve as the basis for the Ayurvedic medical system in India. It is often referred to as "Margosa" or "Indian lilac".Azadirachta indica tree has been ranked among the top ten plants by the International Scientific Community to be researched and utilized for the health of all living things and the sustainable growth of the earth. Azadirachta indica is used in traditional medicine as an antihelmintic, antimalarial, antipyretic, bitter tonic, and for antiviral and antibacterial properties(4,5).

Botanical Description:

Botanical name:	Azadirachta indica, A.Juss.
Family:	Meliaceae.
Synonym:	MeliaAzadirachtalinn.
Common names Sanskrit:	Ravipriya, Vembaka, Nimba, Arishta.
Bengali, Hindi, Panjabi:	Nim or Nimb, Nimgachh.
Gujarati:	Limba.
Tamil:	Vembu.
English:	Neem or Margosa tree; Indian Lilac.
German:	Indischer Zedrach.
French:	Azadiraed'Inde.

Active Ingredients:

Since it is a rich source of many different kinds of chemicals, Azadirachta indica L.or neem, exhibits therapeutic importance in health management. Azadirachtin is the most significant active ingredient; the others are quercetin, sodium nimbinate, gedunin, salannin, nimbin, nimbidin, and nimbidol. Ascorbic acid, n-hexacosanol, amino acid, nimbin, nimbanene, 6-desacetylnimbinene, nimbandiol, nimbolide, 7-desacetyl-7-benzoylazadiradione, 7-desacetyl-7-benzoylgedunin, 17-hydroxyazadiradione, and nimbiol are present in leaves. Polyphenolic flavonoids such as quercetin and ß-sitosterol were extracted from fresh neem leaves and shown to possess antimicrobial and antifungal characteristics.

Process of Wound Healing:

The process by which a living thing replaces lost or injured tissue with newly formed tissue is known as wound healing. The epidermis, which is the skin's surface epithelial layer, and dermis, which is the skin's deeper connective layer, work together to create a barrier that shields healthy skin from the elements. A controlled series of biochemical processes are triggered when the barrier is breached in order to heal the harm. Haemostasis (blood clotting), inflammation, tissue growth (cell proliferation), and tissue remodelling (maturation and cell differentiation) are the typical stages of this process. Rather than being a distinct stage, blood clotting could be viewed as a component of the inflammatory stage (6).

Types of injuries:
Damage to the integrity of biological tissue, such as skin, mucous membranes, and organ tissues, is the broad definition of a wound. Open and closed wounds are two different categories of wounds:

Open Wounds: These injuries have split or broken skin, exposing the underlying tissues to the external environment.

Closed Wound: The skin's surface remains unharmed, but there may be damage to the underlying tissues.

Wound Healing Stages:
The four continuous and overlapping phases of haemostasis, inflammation, proliferation, and remodelling should be involved in optimum wound healing(7).

Phase of Haemostasis (blood clotting):
Takes place extremely rapidly. It begins with blood seeping from the body, and as a result, blood vessels narrow (Vasoconstriction) and limit blood flow. In a matter of seconds after the blood vessel's epithelial wall ruptures, the platelets clump together and stick to the sub-endothelium surface. After that, in roughly sixty seconds, the first fibrin strands start to stick together. Pro-coagulants and the release of prothrombin cause the blood to change from liquid to gel when the fibrin mesh begins(8,9). The platelets and blood cells remain trapped in the wound area due to the formation of a thrombus or clot.Generally speaking, the thrombus is significant throughout the healing stages of a wound; however, it becomes problematic if it separates from the arterial wall and enters the bloodstream, potentially leading to a heart attack, stroke, or pulmonary embolism.

Phase of Inflammatory:
Starts as soon as the damage occurs, resulting in localized swelling from the ruptured blood vessels' transudate leakage (a mixture of protein, salt, and water).Inflammation inhibits bleeding as well as shields against infection. Cells that promote healing and repair might migrate to the site of the wound thanks to fluid engorgement. Damaged cells, infections, and bacteria are eliminated from the wound site during the inflammatory phase. The swelling, heat, pain, and redness that are frequently observed at this stage of wound healing are caused by white blood cells, growth hormones, nutrition, and enzymes. Only when inflammation is severe or protracted can it become an issue in the healing process of a wound.

Proliferative Stage (the development of new tissue):
When new tissue composed of collagen and extracellular matrix is used to rebuild the wound. The incision shrinks as new tissue grows. In order to ensure that the granulation tissue is healthy and gets enough oxygen and nutrients, a new blood vessel network must be built. By grasping the borders of the wound and drawing them together with a process akin to smooth muscle cells, myofibroblasts cause the wound to contract(9,10). Granulation tissue has an irregular texture and is pink or crimson in the good stages of wound healing. Granulation tissue in good health does not bleed readily. An infection, ischemia, or inadequate perfusion may be indicated by dark granulation tissue. At last, the damage is resurfaced by epithelial cells. Maintaining moisture and hydration in wounds speeds up the process of

epithelialization. Generally speaking, occlusive or semi-occlusive dressings will maintain the proper tissue humidity to maximize epithelialization if they are administered within 48 hours after the injury(11,12).

Phase of Maturity (Remodelling Stage):

The wound heals completely and collagen changes from type III to type I. Apoptosis, or planned cell death, eliminates the cells that were utilized to heal the wound but are now no longer required. The wound is thick and the collagen that was deposited during the proliferative phase is haphazard. Along stress lines, collagen is rearranged into a more ordered structure, which strengthens the mending tissues' tensile strength. Matrix metalloproteinases are released by fibroblasts. Type III collagen can be remodelled into type I collagen more easily thanks to the enzymes. Remodelling typically starts 21 days following an accident and lasts for up to a year. Healed wound areas often only have 80% of the tensile strength of uninjured skin, and even with cross-linking, they remain weaker than uninjured skin(13).

Procedure:

Chemical Requirements:

Methyl paraben as a preservative agent collect from pharmaceutics laboratory, BPS, Chitosan as film forming agent collect from local market,Suri ,Nutrient agar culture media was collected from Nice Laboratory and Staphylococcus aureus sample collected from Central laboratory Kolkata. Methanol is collected from RSS Distributor.

Extraction of neem:

A. First we accumulated neem leaf and wash that by way of regular water for elimination of all the impurities that are gathered to neem leaf. B. After washing the neem leaf are dried over warm air oven at 50-60° C for seventy two hr for complete drying. C. Grinding dried neem leaf with mortar pestle. D. After that we weighted 35 gm of dried neem leaf powder and combined it to the in the past organized 100ml 95% electricity of ethanol and remain it in room temperature for 7 days with often stirring with glass rod. G. Filtered the combination the use of a filter paper and Collect the filtrate liquid in a beaker. H. Cover the beaker with aluminium foil to keep away from the solvent ethanol from escaping the mixture.

Extraction of marigold:

A. First we accumulated marigold flower and wash that by way of regular water for elimination of all the impurities that are gathered to marigold flower. B. After washing the marigold flower are dried over warm air oven at 50-60° C for seventy two hr for complete drying. C. Grinding dried marigold flower with mortar pestle. D. After that we weighted 35 gm of dried marigold flower powder and combined it to the in the past organized 100ml 95% electricity of ethanol and remain it in room temperature for 7 days with often stirring with glass rod. G. Filtered the combination the use of a filter paper and Collect the filtrate liquid in a beaker. H. Cover the beaker with aluminium foil to keep away from the solvent ethanol from escaping the mixture.

Preparation of Chitosan disk:

Using a magnetic stirrer, 2% (w/v) of CHT solution was produced in acetic acid solution (1% v/v in DI) and stirred for 24 hours at 60°C before cooling to ambient temperature. Following a Whatman filter paper

filter, the CHT solution was supplemented with a specified amount of GL (0.75 mL per gram CHT) and T (0.2 g per gram CHT). To prepare the films, CHT solution containing GL and T was additionally utilized. Using the magnetic stirrer, the CH components were thoroughly mixed for an additional hour at room temperature. To create the CHT films, these film solutions were put onto glass petri dishes and allowed to dry for 72 hours at room temperature. Every effort was made to get rid of the air bubbles.

Preparation of culture media:
In one liter of distilled water, suspend 40 grams. To fully dissolve, bring to a boil. Use an autoclave set at 121°C for 15 minutes to sterilize. Cool to 45–50°C and aseptically add 6% (5–10% is common) of sterile, defibrinated blood to blood agar.

Development of S. aureus from a frozen stock:
The ideal way to store S. aureus is in frozen stocks at -80°C using a "cryopreservation solution." Starting from a frozen stock, bacteria must first be cultured on solid agar.
Process the transfer of S. aureus from a frozen stock to the culture media-
Put on a protective lab coat, goggles, and disposable latex gloves before working in a biosafety cabinet. Using a sterile loop, remove a tiny amount of bacteria from frozen stocks. Store frozen stocks in a cooler or on ice to reduce temperature fluctuations that could otherwise compromise their viability.
Using the loop, transfer the frozen aliquot of S. aureus onto an agar plate, streaking across the plate from top to bottom and left to right to create isolated colonies. After inverting the plates, incubate them at 37°C for 12 to 16 hours.

Conditions of Growth:
Temperature ranges for S. aureus growth include 15° to 45°C and concentrations of NaCl up to 15%. Prolonged exposures above 42°C or below 10°C are not advised, nevertheless. Plates should not be kept at 4°C for more than a week. S. aureus is resistant to alcohol, detergents, and high osmolality because of its highly cross-linked peptidoglycan

Isolation and identification of Staphylococcus Aureus:
Isolates were incubated for 24-48 hours at 37ºC before being cultivated on nutrient agar. Large (2-4 mm), convex, opaque white, and readily emulsifiable colonies were developed. They were then subcultured with sheep blood on blood agar; the majority of the strains were hemolytic. Gram staining (gram+ve cocci, irregular and grape-like cluster arrangement), hemolysis (beta hemolysis), and coagulase test (+ve) were used to identify the colonies.

Antimicrobial susceptibility testing by Agar cup diffusion method:
This method of testing for antibiotic susceptibility is also known as the Kirby-Bauer method. It is employed to determine a bacterium's antibiotic susceptibility. Since Mueller-Hinton Agar has good repeatability, is ideal for testing antibiotic susceptibility, and allows for the adequate growth of a wide variety of pathogens, it is frequently utilized.

The MHA (Mueller-Hinton Agar) medium's preparation:
Dehydrated medium is made using either distilled or deionized water to yield MHA. After heating it to a

perfect dissolve, it is autoclaved for 15 minutes at 121 degrees Celsius. After every sterilization, the pH was measured; it should be between 7.2 to 7.4. The agar was put onto a sterile petridish at a consistent depth of 4 mm after being cooled to between 40 to 50 degrees Celsius. The plates were dried in an incubator at a temperature between 30 to 37 degrees Celsius until all of the excess moisture on the surface was gone before using. Each of the three bacteria was given its own petridish.

Incorporation of Bacteria onto the MHA medium:

To ensure consistent inoculation, ideal colonies of each bacteria from their different subcultures were swooped with a sterile inoculating loop and streaked over on each of the three MHA media. This process was repeated by rotating the plate 60 degrees. The beginning of the streaking was indicated.

The positioning of the antimicrobial disks:

Neem extraction

Neem extract antimicrobial disks were placed over the inoculation medium using sterile forceps, and they were quickly gently pressed to guarantee full contact between the disk and the agar medium. They were then incubated for 24 hours at 37 degrees Celsius.

Neem extract with floral extraction of Marigold

Antimicrobial disks of neem extract with flower extraction were placed over the inoculation medium and gently pushed with sterile forceps to guarantee full contact between the disk and the agar medium. They were then incubated for 24 hours at 37 degrees Celsius.

Result:-

Sample was taken to look for the zone of inhibition after being left undisturbed for 16–18 hours.

Based on the findings observed in the culture media, neem extract has antibacterial action against gram-positive bacteria (Staphylococcus aureus), with a 26 ±2 mm zone of inhibition. And also Neem extract with floral extraction of Marigold has more antimicrobial activity against gram-positive bacteria (Staphylococcus aureus), with a 34 ±2 mm zone of inhibition.

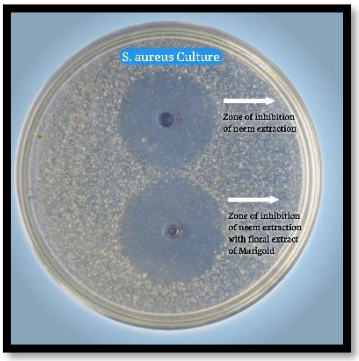

Fig.1 Zone of Inhibition

After the experiment we can see the neem extract with floral extraction have more antimicrobial , antifungal, antiviral and much more activity than only Neem **(***Azadirachta indica)* Extract.

Conclusion:

Neem and marigold both are commonly known as the "miracle leaf" or "life plant," has a rich history of traditional medicinal use across various cultures. Its leaves contain various bioactive compounds, including flavonoids, alkaloids, and phenolic acids, which have sparked interest in its potential therapeutic applications in modern medicine. While traditional uses include wound healing, inflammation reduction, and antimicrobial actions, recent research has delved into its broader pharmacological effects, such as antioxidant properties and potential anticancer activity. Experimental studies have provided promising results, suggesting that neem and marigold extracts may offer benefits in managing conditions ranging from skin ailments to chronic diseases like cancer. However, despite the growing body of preclinical evidence supporting its medicinal properties, several crucial aspects warrant further investigation. First and foremost is the need for rigorous clinical trials to validate the efficacy and safety of neem and marigold preparations in humans. Additionally, research is needed to elucidate the mechanisms underlying its pharmacological effects and to optimize dosage regimens for specific health conditions. Furthermore, as with any herbal remedy, there are considerations regarding quality control, standardization of extracts, and potential interactions with medications. It's essential for individuals considering the use of neem and marigold for medicinal purposes to consult healthcare professionals to make informed decisions. In conclusion, marigold and neem both holds promise as a valuable source of natural remedies, particularly in wound care and inflammatory conditions, its full therapeutic potential remains to be fully understood. Continued scientific inquiry is crucial to unlock the secrets of this fascinating plant and harness its benefits for human health in a safe and effective manner. As we find that clinical trial on the plant yet not done hence the plant can be explored for clinical study.

Appendix:

Neem and marigold also known as the “miracle leaf”. It has a long history of traditional medicinal use in various culture. From this plant exaction the pharmaceutical sector may get various type of medicinal benefit to prepare various type of dosage forms for both internal and external use. This review work ensure that that extraction has the potency to prevent microbial growth and having anti-cancer and anti-diabetic effect also.

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