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Bioprospective Role of *Ocimum Sanctum* & *Solanum Xanthocarpum* against Emerging Pathogen: Mycobacterium Avium Subspecies Paratuberculosis

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Abstract

Mycobacterium avium subspecies *Paratuberculosis* (MAP) chronic, contagious and typically life-threatening enteric disease of ruminants caused by a bacterium of the genus *Mycobacterium* but can also affect non-ruminant animals. MAP transmission occurs through the fecal-oral pathway in neonates and young animals. After infection, animals generate IL-4, IL-5, and IL-10, resulting in Th2 response. Early detection of the disease is necessary to avoid its spread. Many detection methods viz., staining, culture and molecular methods are available and numerous vaccines and anti-tuberculosis drugs are used to control the disease. However, prolong use of Anti-tuberculosis drugs leads to the development of resistance. Whereas vaccines hamper the differentiation between infected and vaccinated animals in an endemic herd. This leads to the identification of plant-based bioactive compounds to treat the disease. Compounds of *Ocimum sanctum* and *Solanum xanthocarpum* have been evaluated to check the anti-MAP activity. Based on the MIC_{50} values Ursolic acid (32-64 µg/mL), Linalool (0.12%), Beta-caryollene (32 µg/mL), Propionic acid (0.25%), Rosmarine acid (1.2 µg/mL), Chlorogenic acid (20-80 µg/mL), Stigmasterol glucoside (0.67 µg/mL), cycloartanol (8 µg/mL), Stigmasterol (3.13 µg/mL), Beta-siyosterol (6.25 µg/mL) were found suitable to act as Anti-MAP.

Keywords: Bioactive compounds; *Mycobacterium avium* subspecies *paratuberculosis* (MAP); *Solanum xanthocarpum, Ocimum sanctum;* Ursolic acid.

Introduction

Johne's disease in ruminants is caused by *Mycobacterium avium* subsp. *Paratuberculosis* (MAP), a persistent rubor with significant economic effects and global dissemination [1]. The apparent correlation between *Mycobacterium avium* subspecies *paratuberculosis* and Crohn's disease in individuals is still being studied extensively, with conflicting results [2-4]. In 1895, German researchers Johne and Frothingham acknowledged MAP for the first time [5]. It commonly infects ruminants (cattle, sheep, goats, deer, and so on) (Figure 1), however, it has also been reported in non-ruminants, notably wildlife [6]. Annual cattle sector losses in the United States have been estimated to be between $250 million [7] and $1.5 billion [8]. According to a new assessment

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of available data employing a Bayesian technique [9], calibrated for susceptivity and explicitness, the underlying frequency of MAP in dairy cattle in the United States was 91.1%, not 70.4% claimed in 2007 [10]. The incidence of MAP in beef cattle herds is 7.9% [11]. Even though JD was initially discovered in the United States during the early 1900s, the emphasis on investigation and disease prevention alone has expanded in the last 20 years. To combat Johne's disease on a farm as well as to recognize herds having minimal infection susceptibility, a discretionary Bovine JD Management Program is in operation. The examination of ambient stool specimens via culturing through elevated sites is among the most cost-effective as well as highly reliable diagnostic techniques for JD [9]. Ironically, wildlife repositories may disrupt initiatives to reduce Johne's disease in livestock unless their significance in wildlife is completely defined [13]. JD transmission is reduced when improved diagnoses are combined with good management strategies [12].

Taxonomy and properties

The *Mycobacterium avium* complex, which belongs to the genus *Mycobacterium* and the family *Mycobacteriaceae*, contains MAP. *Mycobacterium avium* and *Mycobacterium intracellulare* are two distinct species in the *Mycobacterium avium* complex. *Mycobacterium avium* subsp. avium, *Mycobacterium avium* subsp. *hominissuis* (MAH), MAP, and *Mycobacterium avium* subsp. *silvaticum* are the four subspecies of *M. avium*, according to a thorough sequence-based evaluation of the internal transcribed spacer of 16S-23S ribosomal RNA [14,15]. MAP is a gram-positive, acid-fast, rod-shaped intracellular bacteria with a diameter of 0.5 to 1.5 m. The bacterial cell wall is dense and waxy arabinogalactan holds the mycolate and peptidoglycan layers intact. Bacteria is a slow-growing that takes over 20 hrs. to multiply [16]. Efforts to build up MAP in the research lab medium were initially unsuccessful [17], and it was hypothesized that MAP's failure to cultivate *in-vitro* was due to a scarcity of a crucial development factor. Further analysis revealed that MAP could flourish on a medium enriched with extracts from many other mycobacteria [18,19], leading scientists to assume that MAP cannot generate a vital growth factor that some other species can synthesize. Mycobactin is a siderophore that binds iron and is produced from *Mycobacterium phlei*, which has been identified as the growth factor required for MAP cultivation *in-vitro* [20,21]. Mycobactin dependence has been regarded as taxonomic for MAP since that period. A mission in the mbtA gene in the mycobactin-production operon has recently revealed a molecular knowledge of mycobactin reliance, as explained further below with the genome sequence [22,23].

Pathogenesis

Johne's Disease (JD) is characterized by persistent diarrhea and a malabsorption condition, which results in malnutrition and muscle atrophy (Figure 2A). The faeco-oral pathway is the most common way for neonates and young animals to become infected. Milk feeding from infected dam is another source of infection to neonates [24]. Calves up to the age of six months have a greater incidence of infection, but afterward, the risk reduces [25]. According to animal research, M-cells and enterocytes both promote MAP adjunct to and transit through the gut mucosa upon consumption [26]. Tissue culture observations demonstrate that MAP influences the establishment of tight junctions in the intestinal mucosa, offering a mechanism for enhanced permeability [27] (Figure 2). Antigens 85 [28], 35 kDa [29], MAP oxidoreductase [30], MAP fibronectin-binding protein [31,32], and histone HupB [33] are all crucial in MAP epithelial cell adhesion and/or penetration, and there is a lot of host-pathogen interaction going on. Prior literature has shown that phagosome acidification stimulates interleukin (IL)-1 production, macrophage recruitment, and trans-epithelial migration in MAP-infected epithelial cells utilizing the cow mammary epithelial cell line MAC-T [34] and bovine Blood-Monocyte-Derived Macrophages (BMDM) [35]. Bacilli (genus Bacillus) are subsequently phagocytosed in the sub- and intraepithelial spaces by these macrophages [36-38]. For pathogenesis, MAP's capacity to persist and proliferate once inside phagocytic cells is fundamental [39,40]. Furthermore, researchers observed that the lipid content of MAP changes in macrophages that acquire a pro-inflammatory phenotype utilizing a culture passage model (Figure 3) [41].

The pathognomonic granulomatous enteritis of Johne's illness [38], which is characterized by a wide and ridged intestinal wall as well as inflammatory lymph nodes, is the result of the ensuing host cellular immunological response. Toll-like receptors help tissue macrophages and dendritic cells recognize molecular patterns linked with pathogens in the innate phase, as well as the abstraction of cytokine-mediated cellular connections and antigen processing [42,43]. In the acquired immunity phase, Th1 T-helper cell responses and concurrent stimulation of macrophages by Interferon-Gamma (INF) produced by Th1 T cells are used to reduce MAP infections [44,45]. The inferential function of nitric oxide synthase, has already been shown in cattle, is implicated in the killing process of these activated phagocytic cells [46]. In this condition, BMDM recovered from sub-clinically contaminated animals exhibits exceptionally high levels of nitric oxide generation (Figure 4) [47]. MAP, on the other hand, affects the activity of bovine macrophages, as demonstrated by distinct profiles of mRNA expression [48], apoptosis suppression and antigen distribution [49], and diagnostic cytokine expression patterns [50]. In infected bovine T helper cells, MAP mostly generates a Th2 response, with increased production of IL-4, IL-5, IL-10, and tissues remodeling inhibitors [51,52]. This humoral response was confirmed in a newborn calf model [53]. In addition, in both ruminants and animals, regulatory T and Th17 cells have been involved in the immune pathogenesis of JD [49,54].

MAP pathogenesis has been studied using a variety of models. MAP, on the other hand, produces immunological responses in ruminant hosts not found in traditional in vitro models. MAP bacilli grow during 4–8 days in infected BMDM [44,55], although bacterial burdens are reduced over time after infection of the murine J774 macrophage cell line [44,55-57]. When researching, the interactions between MAP and phagocytic cells, it is preferable to use primary phagocytic cells. To follow the progression of MAP infection from initial to final stages, Ileal loops have been employed to establish a prospective systems biology approach [58]. The host transcriptome profile following infection with *M. avium* subsp. *avium* and MAP were recently compared using this paradigm. Intestinal mucosal weakening, activation of a Th2 reaction, and phagocytosis suppression were all related to MAP transmission, which was not found with *M. avium* subsp. *avium* infection (Figure 5) [59].

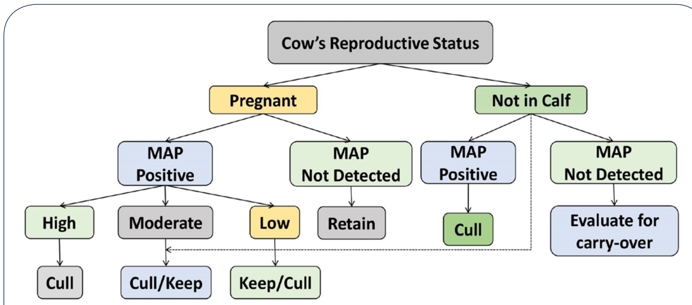


Figure 1: Control of *Mycobacterium avium* subspecies *paratuberculosis* (MAP) infection in domestic livestock.

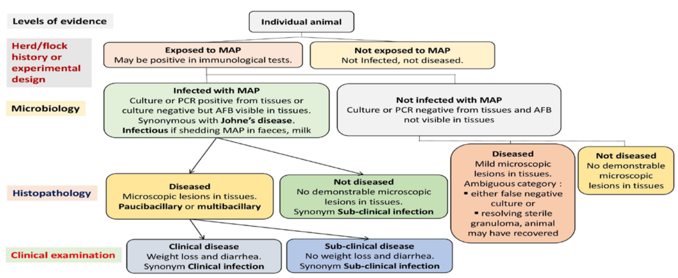


Figure 2: Primary classification of animals exposed to *Mycobacterium avium* subspecies *paratuberculosis* (MAP) using a systematic diagnostic approach.

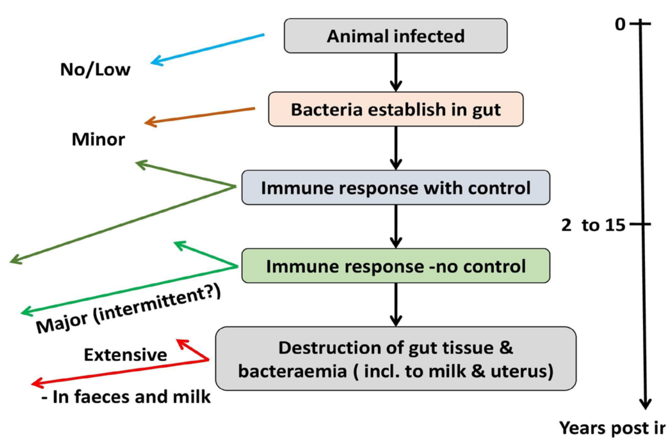


Figure 3: Pathogenesis of MAP infection in cattle.

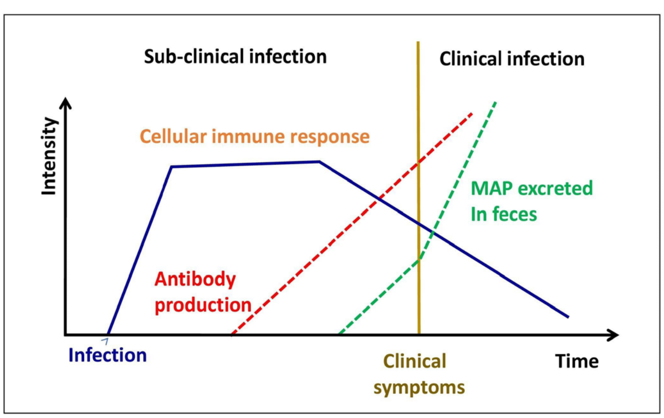


Figure 4: Different stages of MAP infection in domestic livestock.

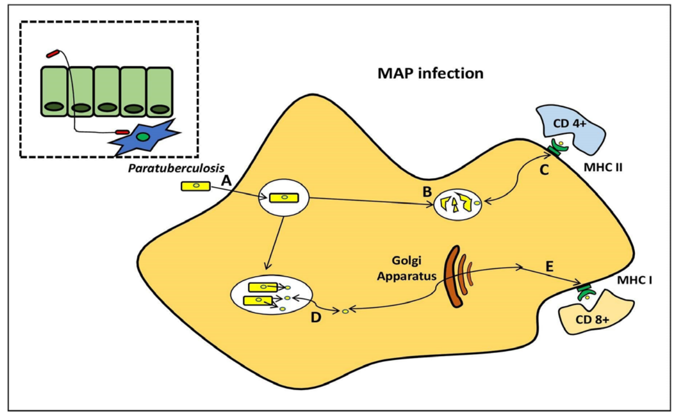


Figure 5: *Mycobacterium avium* subspecies *paratuberculosis* within the macrophage. MAP bacilli passing by the mucosal barrier, preferentially via M cells, and after which it is engulfed by subepithelial macrophage (A). MAP bacilli degraded within the phagosome (B) and also stimulate CD4 + immune responses via antigen presentation through the MHC class II pathway. (C) MAP evades destruction within phagosome, through the inhibition of general killing mechanisms, and proliferates. (D) MAP proteins secreted pass from the phagosome to the cytosol and be subsequently available for presentation to (E) CD8+immune responses via the MHC class I pathway.

Diagnosis and control

Before any clinical indications, infected animals shed MAP in their feces, making them a prominent cause of infection forthe herd's other animals. To avoid the spread of JD, it is critical to diagnose the infection as soon as possible. Based on the detection of MAP both directly and indirectly, many diagnostic tests have been created [60]. Direct identification of MAP in clinical specimens can be thriving using (i) microscopy, (ii) culture-based MAP isolation, and (iii) PCR-based MAP DNA identification. Clinical samples have been analyzed using acid-fast staining or Ziehl–Neelsen. Acid-fast staining is the easiest, quickest, and also a most economical mode of diagnosis, but its accuracy and precision are inadequate since it is challenging to discern between MAP and some other acid-fast bacilli [61]. Although Ziehl–Neelsen staining can also be used to screen for MAP; it must be verified by additional procedures such as PCR and/or immunoassays. The "gold standard" for JD diagnosis is MAP isolation through culture. The fact that MAP requires mycobactin J to grow in a specific laboratory medium can be utilized to distinguish it from many other acid-fast bacteria. A novel growth media that increases MAP restoration and sensitivity by 1,000-fold was recently divulged [62]. Because MAP develops slowly (On solid medium, colony development takes 6–8 weeks.), culture-based diagnosis takes a long period. As a consequence, a highly fast and precise PCR-based test was employed for MAP identification in environmental and clinical specimens [63-65]. IS900 is a 1.4 kb multi-copy insertion element that is sequence specific to MAP. The primers used in this PCR are for IS900 [60, 66]. Other mycobacteria with IS900-like insertion sequences, on the other hand, have been demonstrated to influence the specificity of this test, resulting in false-positive findings [64,67]. To prevent false-positive results, a multiplex PCR centered on the IS900, IS901, IS1245, and dnaJ genes was constructed, although the precision of this assay is restricted owing to reagent interference and primer-dimer generation [60, 68]. Furthermore, PCR tests based on stool specimens hold only 70% sensitivity and 85% specificity [69]. There has been some advancement in identifying and utilizing more precise targets for PCR testing [70-72], and this comparative genomic technique has addressed an apprehension gap in MAP identification. Several of these objectives have made their way into commercial diagnostic tools.

The immunological response of the host to infection is the basis for diagnostic MAP tests based on indirect detection. A Johnin pure protein derivative was used to produce the delayed-type hypersensitivity skin test [73]. However, because various environmental mycobacteria might sensitize the animal and provide false-positive findings, this test is not specific. As a result, delayed-type hypersensitivity skin tests can't tell the difference between vaccinated and animals that have been naturally affected. As previously established, MAP invasion triggers T helper cells, which secrete IFN-γ. The utilization of cultures supernatants from day-old blood specimens treated with Johnin and co-stimulated with human IL-2and/or bovine IL-12 can also be used to diagnose JD using an enzyme-linked immune sorbent test (ELISA) [74]. Unfortunately, cross-reactivity issues arise because in the INF- test, MAP pure proteins analogs are often used as antigens. A potential alternative MAP antigen for the research was L5P, a cell wall lipopeptide, however, the IFN-γ expression was reported to be weaker than that of Johnin [75]. Antibodies in milk and serum from diseased animals are detected using commercial ELISA kits such as (I)) Para Check (CSL/Biocor), (ii) Herd Check M. *paratuberculosis* ELISA (IDEXX Laboratories, Inc.), (iii) ID Screen® *Paratuberculosis* Indirect (ID Screen® *Paratuberculosis* Indirect (ID Screen (IDvet Genetics) and (iv) SERELISA ParaTB (Synbiotic Corp.). In comparison to PCR testing, an ELISA seems to have a lower sensitivity of 50% but a far higher specificity of 99.8% [76,77]. To establish better sensitive immune-based tests for JD diagnosis, additional research is needed to uncover MAP specialized antigens.

Vaccination (the most economical), screening, and improved herd control are all alternatives for avoiding JD, depending on a producer's finances, infrastructure, and operations [78]. However, while JD vaccines can diminish systemic disease and discharge, their effectiveness is minimal, and none of them provides fairly long immunity. In the United States, for instance, Mycopar® (BoehringerIngelheim Vetmedica, Inc.) has been exclusively licensed vaccination for JD in cattle. Unfortunately, since strain 18 of *M. avium* subsp. *avium* was used to make the vaccine [79], but it lacks an ideal antigenic repertoire. In Australia, Silirum® (Zoetis Animal Health), a different bacterin, is being investigated and it has been licensed for restricted usage in cattle. The MAP 316F strain has been heat-killed in this vaccination. This formulation may contain a broader spectrum of antigenic, however utilizing bacteria that have been destroyed by heat, may lower efficacy while improving safety. Both Neoparasec® (Rhone-Merieux) and Gudair® (Zoetis Animal Health) contain the live-attenuated MAP strain 316F and are authorized for usage in goats and sheep. Vaccines that are currently available, on the other hand, are unable to discriminate between vaccinated and infected animals, impairing JD diagnostic testing [80], and strain 316F was created in the 1920s using random depreciation processes (e.g., passages in ox bile) that are currently being examined [81]. Eventually, to successfully manage JD, an elevated vaccination is necessary [82].

Human anti-tuberculosis vaccines of the latest era appear to provide higher protection than subunit vaccines, according to testing results [83]. Because JD is induced by a bacteria called Mycobacterium, potential subunit or bacterin-based vaccines are likely to face a similar situation. The JD Integrative Protocol-Animal and Plant Health Inspection Service's endeavors to establish a consistent vaccination testing program were spurred by this. In a three-phase investigation, investigators from New Zealand and the U.S provided 22 masked live-attenuated immunization candidates to be evaluated in mouse, BMDM, and goat models. Despite the substantial development of animal screening procedures [84], the bulk of the suppressed transposon variants investigated was the first generation and had the Tn5367 transposase, that caused destabilization. Furthermore, unknowns including the ideal immunization path and dose plan could not be determined before the commencement of the experiment. Despite this, crucial information and chemicals were created [80]. It is yet conceivable to design a subunit vaccine that can manage infections by inducing the appropriate humoral immunity [85], specifically against antigens produced by the pro-inflammatory phenotype [86].

However in absence of a vaccine, control of MAP infection in the human population can be accomplished, either by surgical removal of infected intestines or by medicines [87] using anti-tuberculosis drugs, which had limited success [88,89]. The Prolonged use of anti-tuberculosis drugs led to the development of drug resistance to all the existing anti-mycobacterial molecules. Because of the increase in cases of animal and human infections, demand for natural products as an alternative therapy for this chronic incurable disease has increased. This has encouraged researchers to find out bio-active (marker) compounds from plants with pharmacological properties against symptoms exhibited by MAP-infected domestic livestock populations, e.g., chronic progressive inflammation, etc. Earlier studies have suggested that plant extracts have possible feasibility to decreasing induction of TNF-α that can modulate TNF-α mediated inflammatory pathways and may have potential against diseases arising due to chronic inflammation caused by MAP infection (*paratuberculosis* or Johne's disease in animals and Crohn's disease in humans). Plants extracts play a major role as immuno-modulator and immuno-stimulator and can increase or decrease the level of various pro-inflammatory and inflammatory cytokines during chronic inflammation.

The pre-2nd century 'Charaka Samhita' book reported Ayurveda (Indian traditional medicine) Herbal medicinal plants have been used to cure tuberculosis including various ailments. Decoctions, Infusions, Tinctures and macerations of Herbal medicinal plants parts such as fruits and flowers, stem bark, roots, stems, and leaves have been used for traditional treatment for many century's TB by native people worldwide. Even though ethnopharmacological and ethnobotanical studies consider wide use in the treatment of TB, most of them were established still to be therapeutic and safe doses. Most of the research studies have failed to give scientific proof to therapeutic practices and traditional beliefs. Consequently, this work is an endeavor to archive traditionally medicinal plants used to control TB. Contrasting traditional therapeutic systems used to have been applied to cure TB, going from the poorly documented oral Indian medicine to the well-documented Indian, Ayurveda and so on.

Description of *Ocimum sanctum* plant

Taxonomic classification of *Ocimum sanctum* plant

Scientific Name: *Ocimum sanctum*

AyurvedicName: Tulsi

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Lamiales

Family*: Lamiaceae*

Genus*: Ocimum*

Morphology

Tulsi (Ocimum sanctum) is an upright, multi-branched sub-shrub, 300–600 mm (30-60 cm) tall with hairy stems. Leaves color is purple or green; the petioled, with up to 5 cm (2 inches) long and ovate blade and also a slightly toothed margin; the plant fragrant is very strong and Phyllotaxy is decussate. The flowers are purplish and placed in close whorls on elongated racemes [90]. In India and Nepal, three main types of morphotypes are cultivated that is *Ram tulsi* (which is a common type with broad bright slightly sweet green leaves). Purplish green-leaved is less common *in Krishna* or *Shyamtulsi* and *Vana tulsi*is the common in wild [91].

Soil and climate

Ocimum sanctum (Holy basil) plant can be grown in moderately shaded conditions with low oil contents. Waterlogged conditions can cause root rot and growth to be stunted. It well flourishes under high rainfall and humid conditions. The high temperatures and long days have been found favorable for plant growth and oil production. Soil & Manure: Porous, aerated, and well-drained with added organic manure of soil is required for plant growth. Clay & Sticky soil is not good for the plant's roots.

Floral characteristics

Ocimum sanctum plant is a short-lived perennial shrub or small annual, up to 3.3 feet (100 cm) in height. The simple toothed and hairy stems are oppositely entire leaves along with the stem. The scented leaves are purple or green, depending upon the variety. The white tubular or small purple flowers have green or purple sepals and are supported by terminal spikes. The nut-lets fruits and numerous seeds are produced.

Propagation

Ocimum sanctum crop can be propagating through the seeds and sown in the nursery beds. 300 g of seeds are required in one hectare for the sowing. The nursery should be located in partial shade with sufficient irrigation facilities and soil depth up to 30 cm. well organic manure is applied to the soil and prepared to a seed beds size is 4.5 x 1.0 x 0.2 m. As seed quantity is mixed with the sand ratio is 1:4 required for sown in a nursery bed and 60 days advance in the onset of monsoon. The 8-12 days seeds can germinate and transplant seedlings in about 6 weeks during the 4-5 leaf stage.

Distribution

The Holy basil plant is widely distributed throughout India and Central University of Punjab and Bathinda researchers have done research from the study of large-scale phylogeny graphical of this species using chloroplast whole genomic sequencing then team revealed that this holy basil plant originates from North-Central India [92].

Ocimum sanctum is a native herb in India, and also known as 'Tulsi' belongs to the family Lamiaceae. The Hindu religious tradition is sacred and is viewed as perhaps the most significant plant used in Ayurvedic medicine [93]. Tulsi plants grow in abundance around Hindu temples. Found in so many varieties strong like green and a red, pleasant aroma. In the previous decade several scientific shreds of evidence have been reported [94,95,96] at holy basil has been utilized to treat a variety of many critical diseases [97] including asthma, arthritis, heart problems, eye disorders, blood glucose levels, hepato protective, anticancer, anti-fungal,

Table 1: Biological Mechanism between Bioactive constituents with MIC_{50} Values.

S.No.	Bioactive constituents	MIC_{50} Value	Mechanism	References
1	Eugenol	500 µg/ml	Antifungal	Ahmad et al., 2015 [102]
2	Linalool	0.12%	Antimicrobial	Federman et al., 2016 [103]
3	Ursolic acid	32 µg/mL 64 µg/mL	Antimicrobial	Do Nascimento et al., 2014[104]
4	beta-caryophyllene	32 µg/ml 1024 µg/ml	Antimicrobial	Santos et al., 2021 [105]
5	Propionic acid	0.25% 0.125%	Antimicrobial Antifungal	Haque et al., 2009 [106]
6	Rosmarinic acid	1.2 mg/ml 0.3 mg/ml 2.5 mg/ml	Antimicrobial Antifungal Antiviral	Abedini et al., 2013 [107]
7	Apigenin	>4 mg/ml	Antimicrobial	Nayaka et al., 2014 [108]
8	Orientin	500 µg/ml 1000 µg/ml	Antimicrobial	Karpiński et al., 2019 [109]
9	Isothymusin	200 µg/mL	Antimicrobial	https://www.chemfaces.com/natural/Isothymusin-CFN97562.html [110]
10	Vicenin-2	>188µg/mL	Antimicrobial Antifungal	Mohotti et al., 2020 [111]

Table 2: Biological mechanism between bioactive constituents with MIC_{50} values.

S.No.	Bioactive constituents	MIC_{50} value	Mechanism	References
1	Chlorogenic Acid	20 to 80 μg/mL	Antibacterial	Lou et al., 2011 [118]
2.	Stigmasterol glucoside	0.67 mg/ml	Antibacterial	Swain and Padhy et al., 2015 [119]
3.	3,4-dihydroxy cinnamic acid methyl ester	50- 200 μg/mL	Antibacterial	Hua Du1 et al., 2009 [120]
4.	Solasodine	62.5 μg/mL	Antibacterial	Sinani and Eltayeb et al., 2017 [121]
5.	Solanine	240μg/mL 120μg/mL 90μg/mL	Antifungal Antiviral Antibacterial	Kumar P et al., 2009 [122]
6.	Cycloartanol	8 μg/mL	Antibacterial	Woldemichael et al., 2004 [123]
7.	Stigmesterol	3.13μg/mL 6.25 μg/mL	Antibacterial	Mailafiya et al., 2018 [124]
8.	Beta-Sitosterol	6.25 μg/ml 12.5 μg/ml	Antibacterial	NWEZE et al., 2019 [125]
9.	Apigenin	> 4 mg/mL	Antibacterial	Nayaka et al., 2014 [126]
10.	Esculestin	192 mg/mL <0.015625 μg/mL	Antibacterial Antifungal	Pushpanathan M et al., 2013 [127]
11	Esculin	2500 mg/L 625 mg/L	Antibacterial Antifungal	Mokdad-Bzeouich et al., 2014 [128]
12.	Scopoletin	50 μg/mL (without sorbitol) >200 μg/mL (with sorbitol)	Antifungal	Lemos et al., 2020 [129]

antimicrobial, chronic fever, anti-fertility and bronchitis [98,99] (Table 1) *Ocimum sanctum* have in so many chemical constituents such as carvacrol, eugenol, limatrol, linalool, ursolic acid, caryophyllene, propionic acid, methyl carvicol, Rosmarinic acid, Apigenin, cirsimaritin, Orientin, isothymusin and Vicenin [Figures S1, S2]. Previous research also showed that the Tulsi leaf juice shows complete growth inhibition of Anti-viral and Anti-Mycobacterial activities [100,101].

Description of *Solanum xanthocarpum* Plant

Taxonomic classification of *Solanum xanthocarpum*plant

Scientific Name: *Solanum xanthocarpum*

AyurvedicName: Kantakari

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Solanales

Family*: Solanaceae*

Genus:*Solanum*

Morphology

Solanum xanthocarpum plant is a very thorny diffused bright green perennial herb, at the base is woody. Branches are several and spreading on the ground, the new branches are covered with dense stellate tomentum, yellow, straight, glabrous, prickles compressed, shining often exceeding and 13 mm long. Leaves are 50-100 x 25-57 mm, bearing stellate hairs on both sides of beneath, ovate or elliptic, Petioles are 13-25 mm long. Sometimes becoming nearly glabrous with age.

Soil and climate

Solanum xanthocarpum is a hardy plant mainly grown in tropical and sub-tropical regions. It does adequately over light humus-rich, silty sand to rich loamy soils having pH of 7.0-8.0. Kantakari is a warm-season crop and also a crop grown over saline lands. The most favorable temperature range is 21-27^{o}C for its growth and reproduction. Generally, abundant sunshine is required and dry weather with a long period of warm. In northern India, from December to January in this season the crop is adversely affected due to frost as it causes injury to vegetative parts and in the spring season plant will be recovered.

Floral characteristics

Kantkari flowers are axillary bud, cymes and bluish-violet. The curvedhairy stellate with short pedicels, linear-lanceolate, globose, prickly outside and lobes are 1.1 cm long. Purple Cololla, lobes deltoid, 20 mm long, acute, hairy outside. 1.5 mm long of Filament, 8 mm long of anthers, glabrous, oblong-lanceolate and tiny pores are opening. Style glabrous and ovary is ovoid. The berry-shaped fruits, 13-20 mm in diameter, are white or yellow with green veins and the calyx is enlarged. Seeds are 2.5 mm in diameter, sub-reni form, glabrous, smooth and yellowish-brown

Distribution

Kantakari plant is widely distributed throughout India. The dry situation in the Himalayas as weed ascended to 1500 meters. Abundant by roadsides and wastelands, mainly in Uttar Pradesh, Rajasthan, Madhya Pradesh, Gujarat and Haryana.

Propagation

The crop is elevated by seed and Yellowish-brown color in seeds, small size i.e. 0.25 cm in diameter and glabrous. There is no dormancy period for seeds and can be sown after some days of harvesting. The germination percentage is around 60-70% and it will take 10-15 days to germinate.

Solanum xanthocarpum is a native herb of India, and also known as kantkari belongs to the family Solanaceae. It is a thorny, bright green, perennial plant with woody roots that grow to a height of 2 to 3 meters and is found all over India, primarily in arid regions as a weed on highway shoulders and waste lands. The 1.3 cm in diameter, yellow or white berry with green veins, and expanded calyx-shaped fruits are produced [112]. In the previous decade much scientific evidence has been reported [113] at kantkari has been utilized to treat a variety of many critical diseases including cough, fever, heart diseases, antipyretic, hypotensive, antiasthmatic, antitumor, anti-anaphylactic, aphrodisiac activities, wound healing, anti-inflammatory, urinary bladder, laxative [114], blood glucose levels, hepatoprotective, anticancer, antifungal, antimicrobial, chronic fever, antifertility and bronchitis [115] (Table 2) *Solanum xanthocarpum* have in so many chemical constituents such as chlorogenicacids, stigmasteryl glucoside, glucoalkaloidsolanocarpine, isochlorogenic, carpesterol, methyl ester of 3,4-dihydroxycinnamic acid,neochlorogenic cholesterol, 3,4-dihydroxycinnamic acid (caffeic acid), solanine-S, solasodine, Quercetin 3-O-D-Glucopyranosyl-(1,6)-D-Glucopyranoside, solasonine, Sitosterol-beta-D-Galactoside, solasurine, solamargine, cycloartanol, sitosteryl-glucoside, campesterol, stigmasterol (fruit); sitosterol, flavonal glycoside, apigenin (flower); amino acids and solanocarpine (seeds); esculetin, coumarins, esculin, scopolin and scopoletin (leaves, fruits and roots); norcarpesterol, tomatidenolandcarpesterol (plant) [116] (Figures S3, S4, S5). Previous researches also showed that the kantkari fruit juice show complete growth inhibition of Anti-viral (HIV), anticancer and Anti-Mycobacterial activities [117].

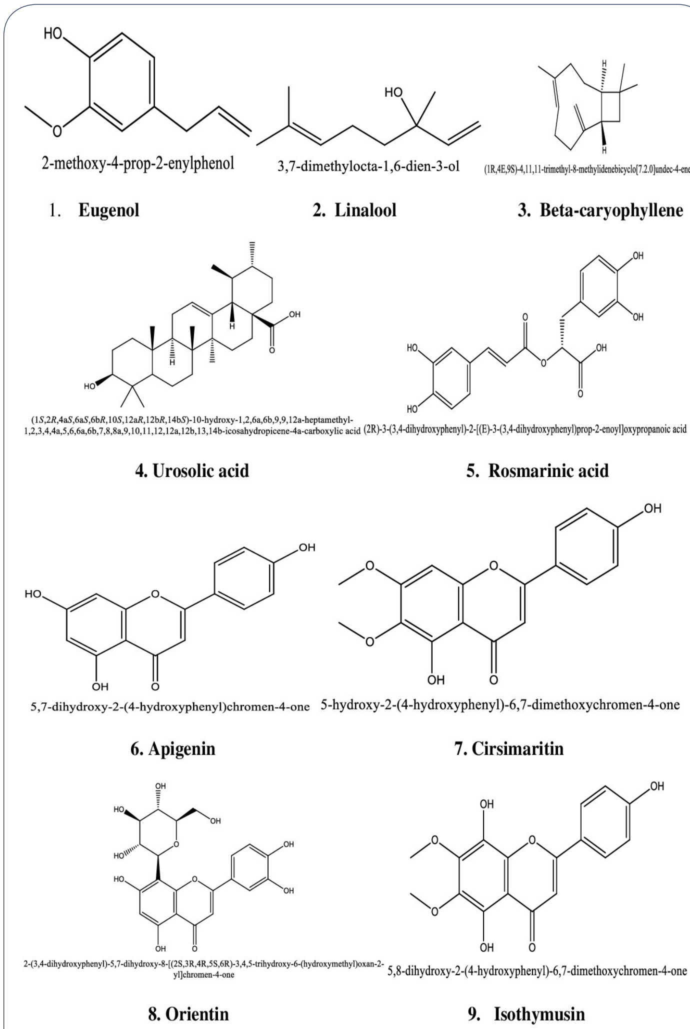


Figure s1: Structures and IUPAC names of 1-9 bio molecules of *Ocimum sanctum* plant.

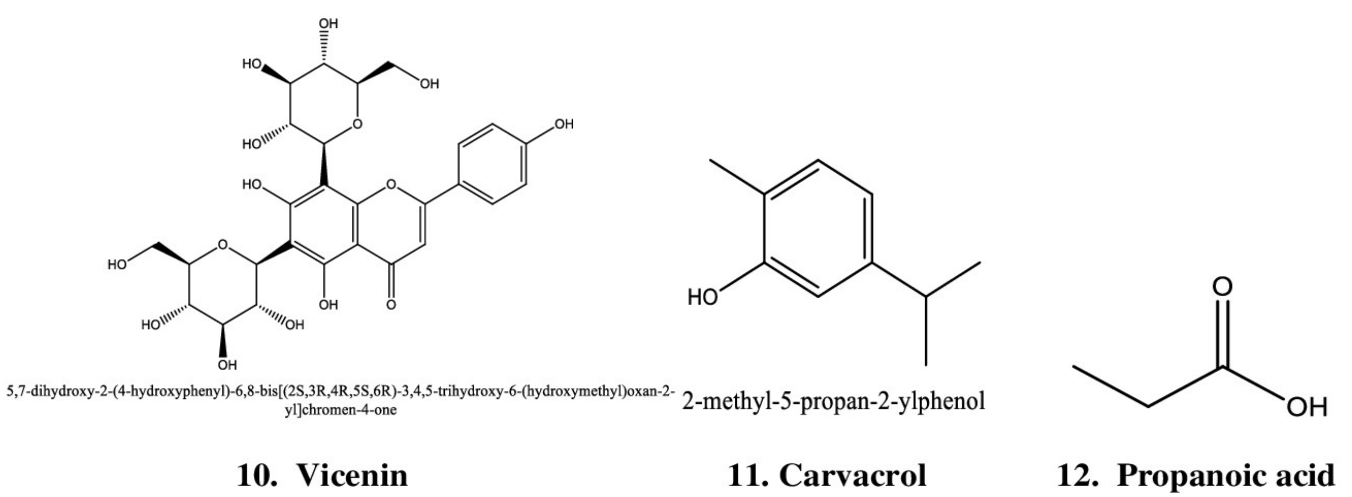
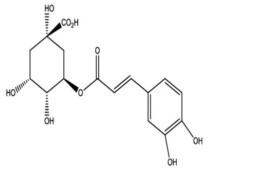
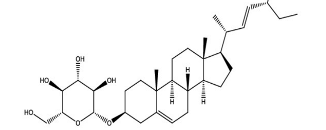


Figure s2: Structures and IUPAC names of 10-12 bio molecules of *Ocimum sanctum* plant.



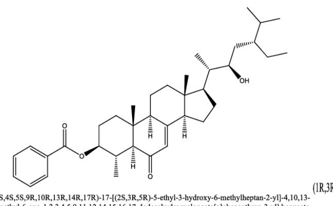
(1S,3R,4R,5R)-3-(((E)-3-(3,4-dihydroxyphenyl)acryloyl)oxy)-1,4,5-trihydroxycyclohexanecarboxylic acid

1. Chlorogenic acid (CGA)



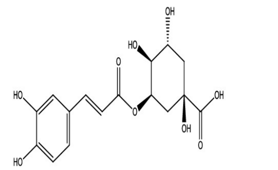
(2R,3R,4S,5S,6R)-2-[[(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol

2. Stigmasterol glucoside



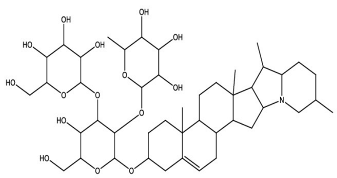
[(3S,4S,5S,9R,10R,13R,14R,17R)-17-[(2S,3R,5R)-5-ethyl-3-hydroxy-6-methylheptan-2-yl]-4,10,13-trimethyl-6-oxo-1,2,3,4,5,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-yl] benzoate

3. Carpesterol



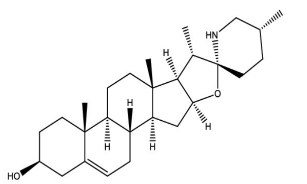
(1R,3R,4S,5R)-3-[(E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy-1,4,5-trihydroxycyclohexane-1-carboxylic acid

4. Neochlorogenic acid



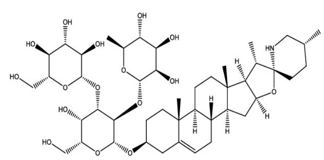
2-[5-hydroxy-6-(hydroxymethyl)-2-[(10,14,16,20-tetramethyl-22-azahexacyclo[12.10.0.02,11.05,10.015,23.017,22]tetracos-4-en-7-yl)oxy]-4-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-3-yl]oxy-6-methyloxane-3,4,5-triol

5. Solanine



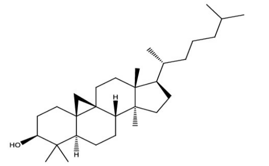
(1S,2S,4S,5'R,6R,7S,8R,9S,12S,13R,16S)-5',7,9,13-tetramethylspiro[5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icos-18-ene-6,2'-piperidine]-16-ol

6. Solasodine



(2S,3R,4R,5R,6S)-2-[(2R,3R,4S,5S,6R)-5-hydroxy-6-(hydroxymethyl)-2-[(1S,2S,4S,5'R,6R,7S,8R,9S,12S,13R,16S)-5',7,9,13-tetramethylspiro[5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icos-18-ene-6,2'-piperidine]-16-yl]oxy-4-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyoxan-3-yl]oxy-6-methyloxane-3,4,5-triol

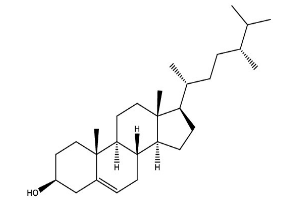
7. Solasonine



(1S,3R,6S,8R,11S,12S,15R,16R)-7,7,12,16-tetramethyl-15-[(2R)-6-methylheptan-2-yl]pentacyclo[9.7.0.01,3.03,8.012,16]octadecan-6-ol

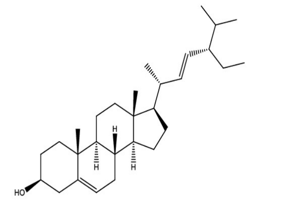
8. Cycloartanol

Figure s3: Structures and IUPAC names of 1-8 bio molecules of *Solanum xanthocarpum* plant.



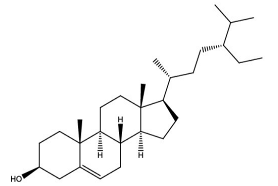
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

9. Campesterol



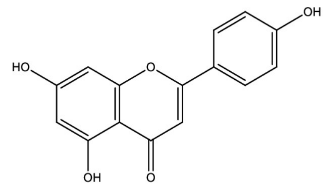
(3S,8S,9S,10R,13R,14S,17R)-17-[(E,2R,5S)-5-ethyl-6-methylhept-3-en-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

10. Stigmasterol



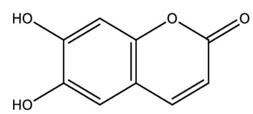
(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol

11. Beta-sitosterol



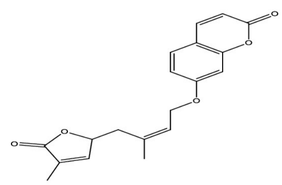
5,7-dihydroxy-2-(4-hydroxyphenyl)chromen-4-one

12. Apigenin



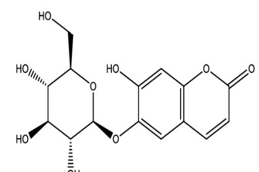
6,7-dihydroxychromen-2-one

13. Esculetin



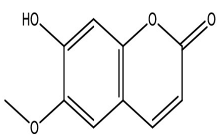
7-[(Z)-3-methyl-4-(4-methyl-5-oxo-2H-furan-2-yl)but-2-enoxy]chromen-2-one

14. Coumarin



7-hydroxy-6-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-2-one

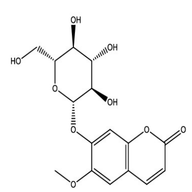
15. Esculin



7-hydroxy-6-methoxychromen-2-one

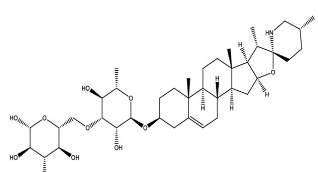
16. Scopoletin

Figure s4: Structures and IUPAC names of 9-16 bio molecules of *Solanum xanthocarpum* plant.



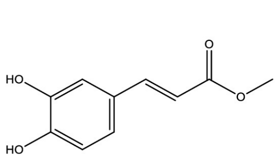
6-methoxy-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromen-2-one

17. Scopolin



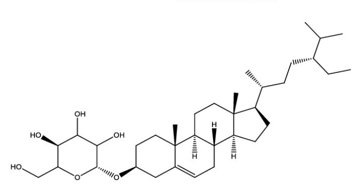
(2R,3R,4S,5S,6R)-6-[[(2S,3S,4R,5R,6R)-3,5-dihydroxy-2-methyl-6-[(1S,2S,4S,5'R,6R,7S,8R,9S,12S,13R,16S)-5',7,9,13-tetramethylspiro[5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icos-18-ene-6,2'-piperidine]-16-yl]oxyoxan-4-yl]oxymethyl]oxane-2,3,4,5-tetrol

18. Solasurine



methyl 3-(3,4-dihydroxyphenyl)prop-2-enoate

19. 3,4-dihydroxycinnamic acid methyl ester



(2R,5S)-2-[[(3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5-ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-yl]oxy]-6-(hydroxymethyl)oxane-3,4,5-triol

20. Sitosteryl-glucoside

Figure s5: Structures and IUPAC names of 17-20 bio molecules of Solanum xanthocarpum plant.

Conclusion

Natural chemicals can be utilized to enhance the efficacy of anti-tuberculosis treatments and perhaps fill in the gaps where regular prescription therapies have lost their effectiveness. Prevention and treatment strategies, combined with natural substances, may be a feasible alternative for reducing drug resistance. As discussed, natural substances possess a multitude of antimycobacterial characteristics and focus on several therapeutic targets. For instance, natural compounds can augment the sensitivity of mycobacterium to antibiotic treatment. Natural items should be researched further for the treatment of active TB. It is worth noting that many of the studies included in this review were carried out using techniques such as molecular assays, mouse models, animal cells, and bacterial culture. Natural products must be of excellent quality, authentic, well formulated, regularly derived from their sources, and not contaminated with other products. Novel natural chemicals are being researched in the hope that they will be effective in treating tuberculosis infections.

We emphasize on identifying plants based on ethnomedical complaints and testing their extracts/phytomolecules against *My-*

cobacterium paratuberculosis strain. In conclusion, we tried to give brief idea about those natural compounds found suitable to paraphrase research activity against *paratuberculosis*. In a result we can say that two plants extract can achieve good combination effect, although any antagonistic effect was not determined yet. Therefore, targeting these two agents will help in future to shorten the current therapeutic regimens for *para* TB and also for treating other tuberculosis diseases also.

Conflict of interest: There is no conflict of interest to declare.

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