



Comparative Assessment of Antibacterial Activity of Secondary Metabolites from Three Medicinal Plants: *Ocimum sanctum*, *Azadirachta indica* and *Mentha arvensis*

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Received date: 30th November 2012, Accepted date: 5th December 2012, Published date: 1st March 2013

Abstract:

Indiscriminate use of antibiotics has ensued into pathogen resistance, therefore, it is essential to discover the natural alternatives to combat the infectious diseases caused by these bacteria, and one scientific approach is to examine the antibacterial activities of the secondary compounds from medicinal plants that are considered to have significant prophylactic properties from centuries. In the present study, three medicinal plants: *Ocimum sanctum*, *Azadirachta indica* and *Mentha arvensis*, have been selected for the extraction of secondary metabolites to perform a comparative evaluation of their antibacterial activities, using Agar-well diffusion method, against three bacterial strains: *Bacillus larvae*, *Actinomyces bovis* and *Staphylococcus aureus*, which have been isolated from soil and identified by Gram staining and biochemical tests. Secondary phytochemicals extracted from *Mentha arvensis* demonstrated the most profound activity against *Staphylococcus aureus* and *Actinomyces bovis*, which indicates that it can be a potential counteractive against these pathogenic bacteria and facilitate the discovery novel. It can be used as a natural antibacterial agent that can combat the serious issue of growing multi-drug resistance among microorganisms.

Keywords: Antibacterial activity, Medicinal Plants, Secondary Metabolites

Introduction:

Emergence of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Because of the side effects and resistance that pathogenic microorganism build against antibiotics, many scientist have recently paid attention to extracts and biologically active compounds isolated from plant species used in herbal medicines^[1]. Every medicinal herb contains a number of active constituents felicitating its manifold curative activity. All of this plethora of compounds with myriad of activities can be roped into animal human system for the same properties. This belief should be the logic of medicinal plants^[2]

Secondary metabolites are nonnutritive plant chemicals that are synthesized to protect the plant from adverse circumstances such as microbial or parasitic attack or harsh weather conditions. A single plant may produce an arsenal of protective chemicals in response

to their lack of motility as well as their stresses and interactions with their environment^[3, 4]. Secondary metabolites are separated into nitrogen compounds (alkaloids, non-protein amino acids, amines, alcalamides, cyanogenic glycosides and glucosinolates) and non-nitrogen compounds (monoterpenes, diterpenes, triterpenes, tetraterpenes, sesquiterpenes, saponins, flavonoids, steroids and coumarins)^[5]. Although plants produce these chemicals to protect themselves, research provides evidence that they can treat humans against infections^[6]. An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable^[7].

The stem and leaves of ***Ocimum sanctum*** (tulsi) or holy basil contain a variety of constituents that may have biological activity, including saponins, flavonoids, triterpenoids, and tannins^[8]. In addition, the following phenolic actives have been identified, which exhibit antioxidant and anti-inflammatory activities: Rosmarinic acid, propanoic acid, apigenin, isothymusin and isothymonin. Two water-soluble flavonoids:^[9] Orientin and Vicenin, have shown to provide protection against radiation-induced chromosomal damage in human blood lymphocytes^[10]. Leaves of ***Azadirachta indica*** (neem) and its constituents have demonstrated antioxidant, immune modulatory, anti-inflammatory, antiulcer, antimutagenic and anti-carcinogenic properties^[11]. Most of these beneficial characteristics were attributed to the presence of phenolic compounds^[12]. The leaves of ***Mentha arvensis***, (mint or pondeena) the common edible aromatic herb, have been described to possess various medicinal properties including antimicrobial properties^[13, 14]. It yields essential oil menthol, a monoterpene, which exert, through their rapid evaporation, slightly anaesthetic, and anodyne local effect^[15, 16]. Menthol's ability to chemically trigger the cold-sensitive TRPM8 receptors in the skin is responsible for the well-known cooling sensation it provokes when inhaled, eaten, or applied to the skin.

There has been a revival of interest in herbal medicines. This is due to increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and the need to discover new molecular structures as lead compounds from the plant kingdom. Plants are the basic source of knowledge of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources.

This worldwide interest in medicinal plants reflects recognition of the validity of many traditional claims regarding the value of natural products in health care. Plants with possible antimicrobial activity should be tested against an appropriate microbial model to confirm the activity and to ascertain the parameters associated with it^[17] so, the present research has been conducted to evaluate the antibacterial activities of *O. sanctum*, *A. indica* and *M. arvensis*, to explore their prophylactic potentials against three pathogens.

Materials and Methods:

Plant Material: Fresh leaves of *O. sanctum*, *A. indica*, and *M. arvensis* were collected from normal fields of Lucknow district, Uttar Pradesh, India; identified and authenticated at BioAxis DNA research centre and by various literatures. Sample leaves were washed thoroughly under running tap water and air dried for 5 days.



Figure: 1. Dried leaves of *Mentha arvensis* (upper left) *Ocimum sanctum* (upper right), and *Azadirachta indica* (below).

Test Microorganisms: Microorganisms were isolated from soil using serial dilution technique, mother plate preparation and by obtaining pure culture. Individual colonies from pure culture were Gram stained and observed under microscope. Isolates were further biochemically characterized and then confirmed using Bergey’s Manual of Determinative Bacteriology.

Solvent Extraction: Secondary metabolites were extracted from air dried, pulverized leaves of *O. sanctum*, *A. indica*, and *M. arvensis* using organic solvent (ethanol) through Maceration procedure^[18] Five grams of air dried leaves of each sample plant were powdered using mortar and pestle. Powdered leaves of each sample plant were placed in 100ml of ethanol. The mixture was incubated in refrigerator for overnight. Mixture was then centrifuged at 6000 rpm and supernatant was collected in fresh eppendorf tubes.

Agar-Well Diffusion Assay: In Agar-well diffusion method a well or reservoir containing test compound at a known concentration is brought into contact with inoculated medium^[19]

As the diffusion of antimicrobial agent progresses microbial multiplication also proceeds. At the point bacterial multiplication proceeds more rapidly than the

drug can diffuse and bacterial cells that are not inhibited by antibacterial agents will be able to continue multiplying. The more susceptible the test organism the larger the zone of inhibition will be^[20]. A loop full of bacterial strains was inoculated into three Nutrient agar plates under sterilized condition. After incubation at 37°C for 24 hours, next day three wells of 9 mm diameter per plate were cut and 20 µl of ethanol extract from each sample plant was poured into the wells. The plates were then incubated at 37°C for 24 hours. The zone of inhibition of test organism around each well was measured in mm^[20] [

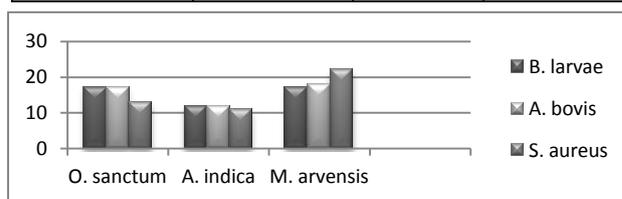
Nutrient agar medium: peptone: 0.5g; yeast extract: 0.3g; sodium chloride: 0.5g; agar: 4g; 100ml distilled water.]

Results and Discussion:

Three bacterial strains, (A) **Bacillus larvae** [gram + bacillus; starch hydrolysis: -; catalase: -; citrate: -], (B) **Actinomyces bovis** [gram + rods; starch hydrolysis: +; citrate: -; glucose fer.: +; Mannitol: +], and (C) **Staphylococcus aureus** [gram + coccus; starch hydrolysis: -; mannitol: +; catalase +; glucose fer.: +], were successfully isolated from soil, characterized and confirmed through several biochemical tests. The antibacterial activity assay was performed using Agar-well diffusion method and diameters of the zone of inhibition have been measured. Among three plants tested, ethanol extract from *Mentha arvensis* exhibited strongest activity against *S. aureus* and *A. bovis*. The largest diameter of zone of inhibition, 22mm and 18 mm, with *M. arvensis* is noted against *S. aureus* and *A. bovis*. Secondary compounds from *O. sanctum* and *M. arvensis* showed equal activity against *Bacillus larvae*, with 17mm diameter of zone of inhibition. The antibacterial activity of secondary metabolites from *A. indica* found to be weakest against all three bacterial strains with zone of inhibition 12mm, 12mm and 11mm, according to Table: 1. Thus, antibacterial activity of *M. arvensis* is “strong” against *S. aureus* and *A. bovis*; *O. sanctum* is “moderate” and *A. indica* is “weak” against all three bacterial strains as far as the comparison is concerned.

Table: 1. Measure of the zone of inhibition in mm.

Sample Plate	<i>O. sanctum</i>	<i>A. indica</i>	<i>M. arvensis</i>
A. <i>B. larvae</i>	17mm	12mm	17mm
B. <i>A. bovis</i>	17mm	12mm	18mm
C. <i>S. aureus</i>	13mm	11mm	22mm



Graph: 1, Indication of zone of inhibition of 3 bacterial species.

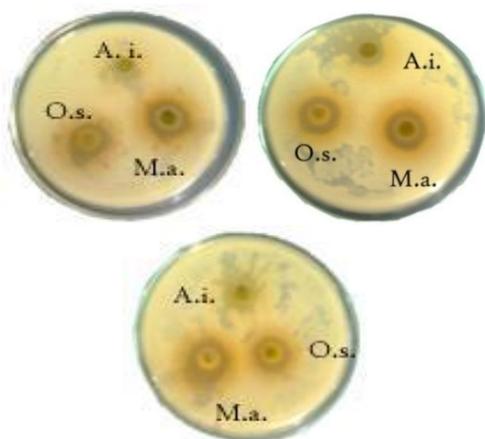


Figure: 2. Zone of inhibition on 3 sample plates; A (left above), B (right above), C (below) [A.i.: Neem, O.s.: Tulsi, M.a.: Mint]

The present study confirms that *M. arvensis* is the most potential source, compared to *O. sanctum* and *A. indica*, to inhibit the growth of common pathogens like *S. aureus*, which is a pyrogenic bacterium, known to play a significant role in invasive skin diseases including superficial and deep follicular lesion and food poisoning [21], and *A. bovis*, cause of common disease of cattle known as actinomycosis, lumpy jaw, and similar human infections [22]. Also, it can be inferred from the findings of the current study that Menthol, which is a terpenoid and a major component of *M. arvensis* [23], may be potentially antagonistic to the pathogens like *S. aureus* and *A. bovis*. Intensive use of antibiotics often resulted in the development of resistant strains [24]. These create a problem in the treatment of infectious diseases; furthermore, antibiotics are sometimes associated with side effects [25] whereas there are some advantages of using antimicrobial compounds of medicinal plants such as, fewer side effects, better patient tolerance, relatively less expensive, acceptance due to long history of use and being renewable in nature [26]. Because of this, the search for new antibiotics from plant origin continues unabated [21]

The ground for the selection of these three medicinal plants for the comparative evaluation of their antibacterial activity is their immense prophylactic potential, which have already been studied and established in prior works. Also, these plants have been a significant source of traditional medicine since ancient times. The Review of the literature shows that various extracts from *A. indica* is beneficial in antipyretic activities [27] and skin ulcers [11]. Clinical studies with the dried neem leaf extract and lotion derived from neem leaf indicated its effectiveness to cure dermatological diseases. [28, 29] It is reported from the review that juice of fresh leaves of *O. sanctum* is given to patients to treat chronic fever [30, 31]. Chewing of *O. sanctum* leaves also cures infections of mouth [32]. Hence, the review of

literature supports the applications of *A. indica* and *O. sanctum* against dermatological diseases, oral infections, and pyrogenic ailments. Nevertheless, these ailments are associated with pathogens like *S. aureus* and *A. bovis* and present comparative study reveals that these pathogens are mostly inhibited by ethanolic leaf extract from *M. arvensis*. Thus, *M. arvensis* should be more effective in treating above diseases than *sanctum* and *A. indica*. Other works are documented that support the findings of the present study, such as, *Sugandhi et al* (2011) [33], shows that antibacterial activity of *M. arvensis* is always highest on *S. aureus* than other bacteria at various concentration of ethanolic extract. *Akram et al* (2011) [34] states that, "Its antibacterial activity against *S. aureus* was highly significant. In this study it was evaluated that *M. arvensis* has antimicrobial activity which justify its use in traditional medicine in infectious diseases [35]". Given the strong antibacterial potential of *M. arvensis* that present study reveals, additional research is required on identification and screening of active agents from *M. arvensis*, as successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development, and computation of minimum inhibitory and bactericidal concentration of plant extract from *M. arvensis* to further exploit this plant species in clinical applications.

Conclusion:

In the present time when multi-drug resistant pathogens are constantly growing and can cause serious clinical implications in the future, scientific exploration on secondary phytochemicals which are naturally oriented to protect plants and contain significant prophylactic properties is the need of the hour, requires discovery of novel and natural antibiotics of plant origin, and which is the hypothesis behind the present study. Results of the present comparative assessment validate *M. arvensis* as a most promising antibacterial agent against *A. aureus* and *A. bovis*, whereas, *O. sanctum* is on the second place and *A. indica* is comparatively weaker in its potential to inhibit all three tested pathogens. In conclusion, *M. arvensis*, due to its profound antibacterial activity, can be used to discover bioactive natural products that may serve as leads for the development of new pharmaceuticals that address hitherto unmet therapeutic needs.

Acknowledgement:

I wish to thank Mrs. Amita Sinha, Head of Academic Operations, BioAxis DNA Research Centre (P) Ltd, for providing me the opportunity of project in the institute.

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