e-ISSN: 0976-822X, p-ISSN:2961-6042

Available online on http://www.ijcpr.com/

International Journal of Current Pharmaceutical Review and Research 2024; 16(2): 47-54

Original Research Article

Formulation and Evaluation of Anti-Microbial Agent in Various Dosage Forms by Using Ethnopharmacological Selected Indian Medicinal Plants (*Solanum xanthocarpum, Adhatoda vasica nees, Oscimum sanctum*)

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Received: 03-01-2024 Revised: 11-01-2024 / Accepted: 23-01-2024
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Conflict of interest: Nil

Abstract

The foundation of the herbal medication business is the ethnomedical values of plants. In order to create herbal medications with minimal side effects, India has contributed its expertise in traditional system medicines (Ayurveda and Siddha). These plants have historically been utilised by tribal communities in India as extracts, powders, or pastes to cure conditions including anti-microbial activity, cough and colds, fever, stomach, kidney, and liver diseases, pain, inflammation, and wounds. The current study focuses on the antibacterial properties of Indian medicinal herbs such Ocimum sanctum, Adhatoda vasica, and Solanum xanthocarpum. Numerous of the long-standing ethnomedicinal applications of these plants are supported by ethnopharmacological investigations. These three ethnomedicinal plants have yielded several phytoconstituents, some of which have exhibited pharmacological activity through in vivo and/or in vitro tests. For herbal medicines to be used safely and effectively, ethnomedical applications that are backed by scientific research are crucial. Using the agar-well diffusion method and various concentrations (30%, 50%, 70%, and 100%), the antibacterial activity of the Solanaceae family plant Solanum xanthocarpum was assessed in vitro against a number of human pathogenic microorganisms, including Escherichia coli, Yersinia pestis, Pseudomonas aeruginosa, and Staphylococcus aureus. In comparison to the control group, chicken flesh that had been washed with AVELE for 90 minutes had higher sensory attribute ratings for juice and tenderness as well as a higher overall sensory quality. This study demonstrates AVELE's ability to kill L. monocytogenes NCIM 24563 effectively, indicating that it may be utilised as a marinade or rinse for meat preservation. The Lamiaceae family of plants includes the fragrant herb Ocimum sanctum Linn, sometimes known as holy basil. It is a native of the tropics of Asia and Africa. To prepare the leaves for grinding, they were detached from the stem, thoroughly cleaned in clear water, and then dried for seven days. In order to create a uniform powder, dried leaves were ground separately in an electric grinder. The powder produced using the "cold extraction method" was converted into ethanol, hexane, and chloroform extract. Ocimum sanctum leaves' antibacterial effectiveness suggests that the plant has strong antimicrobial qualities. Additionally, because ocimum is so common in India, it might be suggested as a conveniently accessible source of antibacterial compounds in place of synthetic chemicals.

Keywords: Ethnomedicinal plants, *Solanum xanthocarpum, Adhatoda vasica, Ocimumsanctum,* Antimicrobial, *Staphylococcus aureus,*Phytoconstituents..

Introduction

Nature has provided us with a diverse range of healing plants. Plants have long been utilised for medicinal purposes in human history. There is a plethora of knowledge, information, and benefits of herbal remedies in our ancient literature of Ayurveda (Traditional Indian Medicine) (Gagandeep et al., 2010). Folk medicine prepared from diverse plants is used to treat common ailments in poor countries, notably India, by low-income persons such as farmers, inhabitants of tiny, rural villages, and members of indigenous tribes (Fabricant and Farnsworth, 2001). India is home to 16 agro-climatic zones, 45,000 plant species, and 15,000 medicinal plants (India Herbs, 2002). Indian medical systems have identified 1,500 medicinal plants, 500 of which are principally used in pharmaceutical production. Three of the ten most popular herbal treatments are available in India, including preparations of Allium sativum, Aloe barbadensis, and Panax spp. (Dubey et al., 2004). Despite its vast medicinal plant flora and fauna, India accounts for just 0.5% of global medicinal plant-related trade exports. According to WHO data, plant extracts are used to treat more than 80%

of the world's population for fundamental ailments. Natural remedies are popular in Asian nations. provides a full overview of past human-plant connections. Traditional medicinal plants have a diverse spectrum of bio-active compounds that are used to treat both chronic and infectious ailments. According to World Health Organisation data, over 80% of people worldwide rely on plant extracts as their primary medical therapy. The usage of herbal treatments in Asian countries exemplifies the long history of interactions between people and plants.

Not just in India but all throughout the world, the usage of herbal medicines made from traditional medicinal plants has grown significantly. Native knowledge of Indian medicine has been passed down through the years, primarily within certain areas or tribal tribes. This age-old knowledge comes from conventional Indian medical systems like Ayurveda and Siddha, and it is also gaining popularity in the West. Due to their low cost and seldom side effects, ethnomedicines and herbal medications have become more popular. The World Health Organisation (WHO) has recently recognised the value of traditional medicine in the healthcare sector. In the Ayurvedic and Siddha medical systems, remedies are made from certain plant components and used to cure various diseases. Numerous traditional medicinal plants used in Siddha and Ayurveda medical systems have been the subject of scientific study for almost 30 years. The scientific evaluation of traditional medicinal plants provides alternative medicines that are supported by science, laying the groundwork for the herbal drug business and identifying therapeutic targets in the pharmaceutical sector.

Kantakari, a popular name for *Solanum surattense*, is a member of the Solanaceae family. The plant contains a variety of nutrients, including carbohydrates, fatty acids, tannins, amino acids, alkaloids, phenolics, flavonoids, sterols, saponins, and their glycosides. Since ancient times, the herb has been appreciated for its therapeutic properties. The Ayurvedic medicinal herbs' roots, leaves, branches, blooms, and fruits are all beneficial components. One of the key components of the well-known Ayurvedic remedy "Dasmula Ashva" is its roots (Amir and Kumar, 2004). According to studies, *Solanum xanthocarpum* has qualities that are anti-fertility, anti-pyretic, anti-cancer, anti-allergy, anti-inflammatory, anti-histamine, hypoglycemic, antibacterial, antioxidant, and antifungal (Yoshida and Oudhia, 2006). Adhatoda vasica, often known as Malabar nut, is a perennial plant well-known in Ayurveda for its medicinal effects (Maurya and Singh, 2010; Kaur et al., 2012). The plant may be found all across India, including higher elevations in the Himalayas, as well as Malaysia, Myanmar, Sri Lanka, and Burma. For thousands of years, indigenous peoples have used A. vasica as medicine, particularly to treat respiratory disorders such as coughs, colds, asthma, and bronchitis (Kaur et al., 2012). A. vasica has been discovered to exhibit a variety of biological actions, including anti-inflammatory, anti-spasmodic, anti-bleeding, anti-diabetic, and anti-jaundice effects, according to Maurya and Singh (2010).

Subramaniam et al. (2015) revealed that A. vasica possesses high antibacterial activity against several foodborne pathogenic bacteria, as well as in vivo effectiveness as a food preservative to prevent the development of L. monocytogenes in processed food. This discovery is noteworthy in light of the possible usage of natural antibacterial agents and their use in the present food preservation scenario. The use of antimicrobial coatings or washings can reduce the prevalence of foodborne infections caused by L. monocytogenes in meat products. This study looked at both AVELE's in vitro and in vivo effectiveness against the microbiological load of L. monocytogenes in chicken flesh to establish its overall effectiveness as a rinse on the sensory quality of chicken meat.

The fragrant herb Ocimum sanctum Linn, sometimes known as holy basil, is a member of the Lamiaceae plant family. Its natural range includes tropical Asia and Africa. One of the world's most widely used medicinal plant families, both as a source of food and medicine and as a source of valuable essential oils used as flavours and spices in a range of culinary dishes. Because of the widespread increase in antibiotic resistance among microbiological illnesses, the medical industry urgently needs new medications. However, traditional plants have been shown to be better suppliers of new antibacterial medicines. The majority of Indian flora contains the best natural medicinal supply sources.

Historically, medicinal plants have been the primary source of new antimicrobial medications. These ancient medicinal plants have significantly improved human health. Plants are also known to be a major source of secondary metabolites and essential oils. The goal of this study was to determine the antimicrobial activity of O. sanctum leaf extracts in ethanol, hexane, and chloroform against pathogenic bacteria in order to determine their antibacterial potential.

Materials and Methods

Collection of plant materials: Plant materials, used for the proposed study were procured from Nathimal Rugan Mal, Khari Baoli, Delhi. The identity of plants was confirmed by internal sources. The collected plant material was brought to the laboratory for further analysis.

Processing of Plant Materials

The obtained Solanum xanthocarpum leaves were pulled off the plant and properly cleaned with tap water before being treated with 2% Mercuric chloride. For faster drying, the leaves were split into smaller pieces. For 15-20 days, cleaned leaves were shade-dried. Using a pestle and mortar, the dried plant material was smashed into fine powder. Finally, the fine powder was kept at room temperature in an airtight container. The dried leaf material (50 g) was pulverised in a blender to a coarse powder and steeped separately in an Erlenmeyer flask in 300 ml of ethanol and acetone. For extraction, the flasks were covered with aluminium foil and let to stand for 3-5 days.The extracts were collected and stock solution of conc. 50 mg/ml was prepared.

Adhatoda vasica powder (100 g) was extracted for 3 hours at 65°C with a 20-fold volume of 70% ethanol, filtered using Whatman No. 2 filtered paper, condensed using a vacuum evaporator, and freeze-dry. The generated AVELE was then kept at 20 degrees Celsius for further examination. AVELE's total phenolic content was evaluated using the Folin-Ciocalteau reagent and gallic acid as the standard phenolic component. To summarise, 20L of the extract solution (1 mg/mL) was added to 100 mL of Folin-Ciocalteu reagent, followed by 80 mL of 10% aqueous sodium carbonate 3 minutes later. After 1 hour at room temperature, the absorbance of the resultant blue-colored liquid was measured at 765 nm against a 70% ethanol (200 mL) control.The calibration curve developed using a standard gallic acid solution was used to quantify the total phenolic content as the gallic acid equivalent (GAE). The results were given in mg GAE/g dry mass. A 250 mL beaker was filled with a sample of AVELE and 200 mL of 10% acetic acid in ethanol. The beaker was then covered and left at room temperature for 4 hours. The sample was then filtered and concentrated to one-quarter of its original volume in a water bath.Drop by drop, concentrated ammonium hydroxide was added to the extract until precipitation was complete. After that, the solution was allowed to stand until the precipitate had settled. The precipitate that formed was collected, washed with diluted ammonium hydroxide, and then filtered. The residue was dried and weighed (total alkaloid). The data are given in milligrammes per gramme of dry extract.

The leaves were taken from the stems, rinsed with clean water, and dried until they were dry enough to grind (7 days). Separately, dried leaves were ground in an electric grinder until a homogeneous powder was formed. The powder produced using "the cold extraction method" was extracted with ethanol, hexane, and chloroform. Tulsi (Ocimum sanctum) extract preparation; (a) Ocimum sanctum plant; (b) leaves separated and dried; (c) leaves pulverised to powder; (d) extract produced from 100% ethanol and other solvents.It was then filtered with Whatman filter paper to achieve a clean filtrate. The resulting filtrate was reduced at a low temperature of less than 60 oC to get a solid residue of Ocimum sanctum (Linn.) extract 2. After dissolving 300 grammes of Ocimum sanctum (Linn.) powder in 1 litre of ethanol and another solvent, 18 grammes of extract (residue) was produced, yielding 6% w/v. 1gm of each extract was accurately weighed and reconstituted in 10 ml of respective solvents to create stoke solution in Ef tube. Furthermore, the dilutions were prepared using the appropriate solvents. To obtain 1mg/ml, 10 mg of Standard Gentamycin was accurately weighed and diluted in 10 ml of distilled water. The various dilutions and standards were pipetted into the designated plates. These were incubated for 24 hours at 37°C and 36 hours at 25o C. Following incubation, zones of inhibition were measured in mm (using an antibiotic zone measuring scale) and compared to a standard.

Procurement of Bacteria

The pathogens were revived in nutrient broth and kept at 4°C in nutrient agar slants. L. monocytogenes NCIM 24563, a pathogenic strain isolated from ready-to-eat food (serotype 1/2a), was acquired from the Microbial Type Culture Collection. The strain was developed in BHI broth at 37°C for 24 hours after being kept on BHI agar at 4°C.

Other bacterial strains, such as Salmonella typhimurium ATCC 43174, were received from the FDA and grown on nutritional agar (NA) at 4 C. Test organism collection and stock culture preparation: For the antimicrobial testing, the following strains were collected. Klebsiella pneumoniae was the Gramme negative bacteria, while Staphylococcus aureus was the Gramme positive bacteria. The Microbial Type Culture Collection (MTCC) in Chandigarh provided all of the microbial strains.

The medium for nutrient broth was made and autoclaved. Following that, the bacterial cultures were injected into separate flasks and shaken for 24 hours.

Screening the Antibacterial Activity of Methanolic and Acetone Extracts of *Solanum xanthocarpum, Adhatoda vasica, Ocimum sanctum*

The agar-well diffusion technique was used to screen leaf extracts (methanol and acetone) of Solanum xanthocarpum, Adhatoda vasica, and Ocimum sanctum. Throughout the study, nutrient agar medium (Beef extract 1g, Yeast extract 2g, Sodium chloride 1g, Peptones 5g, Agar 20g, Distilled Water 1000 ml) was employed. The

medium was autoclaved for 30 minutes at 121.6°C before being put into Petri plates. Bacteria were cultured for 24 hours in nutrient broth. Each nutrient agar plate received 100l of bacterial suspension. In each Petri plate, 8 mm diameter agar wells were created using a sterilised stainless steel cork borer. Each plate's wells were filled with 30%, 50%, 70%, and 100% concentrations of Solanum xanthocarpum produced extracts.

The well in the Petri plate used as a control contained just pure solvent. In the incubation chamber, the plates were incubated at 3720C for 24 hours. The diameter of the inhibition zone surrounding the well (in mm) including the well diameter was used to compute the zone of growth inhibition. For all three replicates, readings were obtained in perpendicular directions, and the average results were tabulated. Percentage inhibition of bacterial microorganism growth was estimated by subtracting control from inhibition diameter values using control as a reference (Hemashenpagam and Selvaraj, 2010). x100% suppression of development (Control Test/Control) Control = average bacterial colony diameter in control. Test = average bacterial colony diameter in treatment sets (Kannan et al., 2009).

Evaluation and Standardization of Pharmacognostic Parameters

To validate the authenticity of the materials, pharmacognostic examinations involving organoleptic tests (macroscopic) and microscopic inspections were performed.

Studies of Macroscopic characters

Air dried sections of Solanum xanthocarpum, Adhatoda vasica, and Ocimum sanctum were examined for macroscopic examination, which included colour, aroma, and taste.

Determination of foreign organic matter

Medicinal plant materials should be devoid of apparent symptoms of mould or insect contamination, as well as other animal contamination, such as animal excreta. There should be no strange odours, discolouration, slime, or evidence of degradation. It is rare to find marketed plant materials that are completely devoid of hazardous foreign particles or residues.

Macroscopic examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable.

Development of dosage forms

Chewable tablet formulations of polyherbal powder extract were optimised based on physical criteria such as appearance, taste, mouth feel, and odour, as well as dosage form parameters such as granule properties, moisture content, hardness, friability, and chewability. Table 1 depicts several PHE-based chewable tablet compositions.

Table 1: Different chewable tablets formulations of PHE

S. No.	Ingredients	T_1	T_2	T_3	T_4
1	Polyherbal extract*	35	35	35	35
2	Sucralose*	2.1.	2.1	2.1	2.1
3	Lactose*	427.5	-	-	-
4	Mannitol*	-	427.5	-	-
5	Dextrose *	-	-	427.5	-
6	Sucrose*	-	-	-	427.5
7	Light $MgCO_3$*	8.5	8.5	8.5	8.5
8	Menthol*	0.625	0.625	0.625	0.625
9	Talc *	5	5	5	5
10	Colloidal silicone dioxide*	1.3	1.3	1.3	1.3

*All quantities in grams **(**Batch size 500 g)

Evaluation of dosage forms

Evaluation of granules: Bulk Density (g/ml) is determined by measuring the volume of a known mass of powder sample that has been passed through a screen into a graduated cylinder (USP-NF, 2012). Weighed 100 g of granules were passed through 1 mm (#18) screen and filled into a dry 250 ml cylinder without compacting. Unsettled apparent volume (V0) was noted down and bulk density was calculated.

$$\text{Bulk Density} = \frac{\text{M}}{\text{V0-}}$$

Tapped Density

(g/ml) is achieved by mechanically tapping a measuring cylinder containing a powder sample (USP, 2012). After observing the initial volume, the cylinder was mechanically tapped, and volume readings were taken. 100 g of granules were weighed and passed through a 1 mm (#18) screen before being put into a dry 250 mL cylinder

without compacting. The cylinder was tapped 500 times on the mechanical tapped density tester to get the tapped volume (Va). The tapping was done 750 times more, and the tapped volume (Vb) was recorded as the final tapped volume.

$$\text{Tapped Density} = \frac{\text{M}}{\text{Vf}}$$

Compressibility index and Hausner ratio

These are measurements of granule compression proclivity (USP-NF, 2012). The compressibility index and Hausner ratio of granules were calculated by measuring both their bulk density and tapped density.

$$\text{Compressibility Index} = \frac{\text{Tapped density} - \text{Bulk density}}{\text{Tapped density}} \times 100$$

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Angle of repose

30 g of granules were weighed and fed through a funnel with a set height (7 cm) on graph paper. Triplicate tests were used to determine the diameter of a heap produced on a horizontal surface and compute the average diameter (d).

$\theta = \tan^{-1}(2h/d)$
Whereas, h is the height of the heap
d is the diameter of heap

Average weight (g)

Ten tablets were selected randomly and weighed.

Diameter (mm)

Digital vernier callipers were used to measure the diameter of the tablets. Ten tablets were chosen at random, and the diameter of each was measured to determine the average diameter (Banker & Anderson, 1991).

Thickness (mm)

It was determined using digital vernier calipers. Ten tablets were selected and the average thickness value was calculated (Banker & Anderson, 1991).

Hardness (N)

A digital hardness tester was used to determine the hardness of the tablets and average hardness was calculated (Banker & Anderson, 1991).

Weight variation

Individually, 20 tablets were weighed and the average weight was calculated. Each tablet weight is compared with average weight as per the specifications given in Indian Pharmacopoeia (IP, 2010).

Friability (%)

Ten pills were weighed and loaded into the Roche Friabilator. Rolling and repetitive shocks from free falls within the device were applied to the tablets. The pills were subtracted after 100 rotations and weighed again (USP, 2012).

$$\text{Friability (\%)} = \frac{\text{Initial wt.} - \text{Final wt.}}{\text{Final wt.}} \times 100$$

Moisture content (%)

Moisture content was determined in chewable tablets formulations as per standard method described in above mentioned section named "moisture content".

Microbial content determination

Microbial content and pathogens in chewable tablets formulation were determined as method described in Appendix 2.4 (API, 2007).

Antimicrobial studies

Antibacterial activity investigations

Media

Mueller Hinton stock gelled by the expansion of 2% agar (bacteriological grade)

Ingredients

Table 2:

Casein enzymatic Hydrolysate	17.5 g/Lt.
Beef Infusion	300 g/Lt.
Soluble starch	105 g/Lt.
Final PH at 25^0C	7.4±0.2

Method of preparation

The above-mentioned raw ingredients were thoroughly mixed with refined water using heat, and the pH was adjusted between 7.2 and 7.6 by using weakening salt or weakening acids.

Sterilization

Test tubes containing 15-20 mL of Muller-Hinton agar and packed with non-retentive cotton were placed in an autoclave at 121°C and pressed factor 15 psi for less than 15 minutes.

Organisms used

Staphylococcus aureus MTCC 7443, Staphylococcus epidermis MTCC 1133, Pseudomonas aeruginosa MTCC 2449, and Escherichia Coli MTCC 1235 were obtained from the Department of Biotechnology at the S.D. School of Pharmacy in Barnala. Gram's staining approach and biochemical reactions were used to validate the equality and nature of the lifeforms. The selected strains were kept frozen by being sub-refined on agar inclines on a regular basis. Following standardisation, a fresh 24-hour stock culture was used for testing.

Working conditions

The entire procedure was carried out under aseptic circumstances using a horizontal laminar air stream equipment. The supplement agar plate was used for air testing and was exposed to aseptic conditions inside the hood prior to beginning the workout hatched to check bacterial proliferation. The lack of microbial lifeforms validates the aseptic working environment.

Preparation of inoculum

The inoculum was prepared fresh in Mueller Hinton stock from the stored frozen inclination culture, incubated at 37oC for 24 hours, and used following standardisation. The test group consists of various concentrations of polyherbal extract, dimethyl sulphoxide as a carrier, and Ciprofloxacin (10 g/circle) as a typical antibacterial medicine.

Antibacterial screening by the Kirby-Bauer method

Before inoculation, 5-6 mm Mueller Hinton agar plates were placed aseptically, allowed to set in an upset posture, and dried at 37ºC. The wet plates were injected with live creatures. Inoculate using a sterile swab. To remove the excess inoculums, the swab was completely turned against the sidewalls of the culture cylinders.

The swab was streaked many times around the outside of the medium, and the plates were rotated at 60o on each occasion. To deal with uniform appropriation of the drug, a sterile circle containing test medication, standard, and vehicle was placed on the exterior of the Muller Hinton agar plates and stored in a chiller for one hour. Three arrangements of plates were arranged and afterward hatched for 18-24 hr at 37ºC. Zones of hindrance for each test sample were estimated in diameter and compared with standard. Each test sample was tried for antibacterial activity against gram positive and gram negative microorganisms (Sahoo et al., 2010).

Result and Discussions

The current investigation found that methanolic and acetone leaf extracts of Solanum xanthocarpum, Adhatoda vasica, and Ocimum sanctum were effective antibacterial agents. Methanolic extracts of S. xanthocarpum, Adhatoda vasica, and Ocimum sanctum inhibited test bacteria growth significantly at varied doses (30%, 50%, 70%, and 100%) as compared to the plant's acetone leaf extract. The methanolic extract of Solanum xanthocarpum, Adhatoda vasica, and Ocimum sanctum was found to be most effective against S. aureus at (18mm at 100%) followed by (15mm at 70%), (13mm at 50%), (11mm at 30%), and it offered minimum inhibition in P. aeruginosa (13mm at 100%), (11mm at 70%), (9mm at 50%) and (9mm at 30%) as given in table 2.

As shown in table 3, the acetone extracts of Solanum xanthocarpum, Adhatoda vasica, and Ocimum sanctum were most effective against S. aureus (16mm at 100%), followed by (14mm at 70%), (13mm at 50%), and (10mm at 30%), and showed the least inhibition against P. aeruginosa (12mm at 100%), (11mm at 70%), (9mm at 50%), and (Nil at 30%). The results showed that methanolic and acetone leaf extracts of S. xanthocarpum, Adhatoda vasica, and Ocimum sanctum were highly successful in preventing the development of Staphylococcus aureus, which is a major human pathogen that causes infections in wounds.

The presence of alkaloids, phenolics, and flavanoids in the leaves of S. xanthocarpum, Adhatoda vasica, and Ocimum sanctum may explain their antibacterial effect. The majority of phytochemical components are recognised to have therapeutic properties such as antibacterial, antifungal, and antioxidant activity (Sahoo et al., 2010).

These findings are consistent with the work of Salie (1996) and Kannabiran (2009). It has been demonstrated that our study is also congruent with that of Aliero and Afolayan (2006). Our findings agreed with those of phytochemicals derived from the leaves of S. xanthocarpum, Adhatoda vasica, and Ocimum sanctum.

Thus, it serves as an encouragement towards development of new drugs for the benefit of mankind.

Table 3: Percent Inhibition of Growth of Human Pathogenic Bacterial *spp*. at different Concentrations of Methanolic Extract of *Solanum xanthocarpum,* Adhatoda vasica, Ocimum sanctum

Concentration of methanolic extract of Solanum xanthocarpum, Adhatoda vasica, Ocimum sanctum (In %)	Inhibition zone diameter (In mm)			
	S. aureus	*E. coli*	*Y. pestis*	*P.aeruginosa*
Control	NIL	NIL	NIL	NIL
30	11	9	9	9
50	13	10	10	9
70	15	13	13	11
100	18	15	15	13

Each data represents the mean of three replicates

Table 4: Percent Inhibition of Growth of Human Pathogenic Bacterial *spp*. at Different Concentrations of Acetone Extract of *Solanum xanthocarpum,* Adhatoda vasica, Ocimum sanctum

Concentration of Acetone extract of Solanum xanthocarpum, Adhatoda vasica, Ocimum sanctum (In %)	Inhibition zone diameter (In mm)			
	S. aureus	*E. coli*	*Y. pestis*	*P.aeruginosa*
Control	NIL	NIL	NIL	NIL
30	11	9	9	NIL
50	13	10	10	9
70	14	13	11	11
100	17	15	15	12

Each data represents the mean of three replicates

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