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Study on the Phytochemical Analysis and Antioxidants, Antimicrobial Activity of Medicinal Plants against Fish Pathogen

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ABSTRACT
The present study was designed to evaluate the phytochemical screening, antioxidants and antimicrobial activities of different plants extracts. Phytochemical screening revealed the presence of terphenols, phenols, quinone, carbohydrates, flavonoid, tannin, saponion, and also screening total content of flavonoid and tannin ,phenol, and antioxidant capacity are extract the plant part of leaves . The powdered plants are extracting with methanol further fractioned with hexane. The total content of tannin is higher in carciya papaya 1.963, total ascorbic acid content higher in carciya papaya. Free radical scavenging activities are higher in carciya papaya 1.642. The total phenol concentration is higher pomengrate. The total flavonoid content has higher in pomengrate and carciya papaya. The reducing power assays are higher in solanum nigrum 0.765. The microorganisms are isolated from fish pathogen. The antimicrobial activity is higher in pomengrate 19 ±0.1. Thin layer chromatography are used to separate the phytochemicals flavonoid, Caumarrins, alkaloid. After 24 hours plates were incubated and using MTT assay (2, 4 Terphenols tetrazolium). The inhibition areas are changed in pink or violet.

Keywords: *Phytochemicals, antioxidant ,MIC,*
Abbrevation: *TLC-Thin layer chromatography,DPPH-1,1-diphenyl-2-picryl-hydrazyl*

INTRODUCTION

Ayurveda has, for several years, exalted the virtues of the vegetarian diet emphasizing on the benefits of green leafy vegetables. Although greens, like spinach have gained universal acceptance through the vastly popular Popeye, they have always been part of the staple diet recommended by Ayurveda physicians. Greens are rich in fiber and antioxidants and contain natural pigments like lute in that keep several diseases, such as macular degeneration, at bay. Ayurveda recommends that greens be included as part of every individual's daily diet to cure diseases.

For thousands of years, the practice of Ayurveda medicine has alleviated illness and attributed over all positive health. The Indian subcontinent has a rich flora of various plants used in traditional medicinal treatment. The plants contain different bioactive ingredients used to cure disease or relieve pain (Samy *et al* 2008). The medicinal properties of these plants could be based on the antioxidant; antimicrobial effects of different phytochemicals present them. Recently the side effects associated with the use of allopathic drugs have resulted in an increased dement for the phytopharmaceutical products of Ayurveda. (Hedge Chaitra *et al.*,2012)

The plants derived antioxidant s especially phenolichas gained considerable importance due to their potential health benefits. Epidemiological studies have consumption of plant foods containing antioxidant's is beneficial to health because it down regulates many degenerative process and can effectively lower the incidence of cancer and cardiac vascular disease. (Arabshahi, 2007)

Plants derived antioxidants are regarded as effective in controlling the effects of oxidative damage, and hence have had influence in what people eat and drink. The ant oxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes (Shahidi *et al*., 1992). The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides **(**Acharya 2004). Oxidative stress involving enhanced generation of reactive oxygen species (ROS) has been implicated in the etiology of over one hundred human diseases including inflammation, metabolic disorders, cellular aging and atherosclerosis, heart disease, stroke, diabetes mellitus, cancer, malaria, rheumatoid arthritis and HIV/AIDS (Aravind, Bhowmik, 2013) Antioxidants are molecules that are capable of neutralizing the harmful effects of the ROS through the endogenous enzymatic defense system such as the superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) in human system. However, with the increasing damaging environmental factors such as cigarette smoke, uvrays, radiation and toxic chemicals; the endogenous defense system is weakened, resulting to a phenomenal disturbance in the equilibrium status of pro-oxidant/antioxidants reactions in living systems. This situation mediates damage to cell structures, including lipids and membranes, proteins, and DNA.

The medicinal plant selected from the present investigation which included *punicagranatum (pomengrate), Mentha spicata (menthol), carciyapapaya (papaya), solanum nigrum* (Manathakali*), Indian goose berry* (Nellikai) have long been used in folk medicine due to their potential health promoting and pharmacological attributes which mainly ascribed to the presence of antioxidants constituents. Such as Flavonoids, alkaloid, saponins, terphenols, Carbohydrates, Tannin, Phenols, Quinone etc., And also study about antimicrobial activity of plant extracts against fish pathogens.

CARCIYA PAPAYA

Carica papaya Linnaeus, (pawpaw), belongs to the family of *Caricaceae*. Papaya is not a tree but herbaceous succulent plants that possess self-supporting stems. Papaya is a large perennial herb with a rapid growth rate. The plants are usually short lived, but can produce fruit for more than 20years. The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female (Bruce and Peter, 2008). The male trees are uncommon, but sometimes occur when homeowners collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self-pollinated.

Carica papaya plants produce natural compounds (annonaceousacetogenins) in leaf bark and twig tissues that possess both highly anti-tumors and pesticides properties. The high level of natural self-defense compounds in the tree makes it highly resistant to insect and disease infestation (Peter, 2008). *Carica papaya L*. leaf tea or extract has a reputation as a tumors-destroying agent. The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender.

INDIAN GOOSEBERRY

Amla is a *Phyllanthacae* Family. It is an indispensable part of the ayurvedic and unani system with amazing remedial qualities. In

Sanskrit, it is called Amalaki or Dhartiphala (Onions, 1994).

In India, it is common to eat gooseberries steeped in salt water and turmeric to make the sour fruits palatable. There are two varieties of Amla cultivated (gramya) and wild (vanya). The wild amla is small, while cultivated amla is big, smooth and juicy. Chemical composition of the amla fruit contains more than 80% of water. It also has protein, carbohydrate, fiber and mineral and also contains gallic acid which is a potent polyphenol. Vitamin C is important for human beings. The amla fruit is reported to contain nearly 20 times as much vitamin C as orange juice. The edible amla fruit tissue has 3 times the protein concentration and 160 times the ascorbic acid concentration of an apple. The fruit also contains higher concentration of most minerals and amino acids than apples. Amla fruit ash contains chromium, Zinc and copper. It is considered as adaptogenic that improves immunity.

The active ingredient that has significant pharmacological action in amla is designated by Indian scientist as "Phyllemblin". The fruit is rich in quercetin, phyllaemblic compounds, gallic acid, tannins, flavonoids, pectin, and vitamin C and also contains various polyphenolic compounds. A wide range of phytochemical components including terpenoids, alkaloids, flavonoids, and tannins have been shown to posses' useful biological (**Aiyeloja**2006).

MENTHA PIPERITIA

Mentha, a member of the *Labiatae* family is originated from Eastern Asia. Among the two major forms, namely *Mentha piperita*L. and *Mentha spicata* L. *Mentha spicata* is locally known as 'Pudina' in Bangladesh. Its English name is Spearmint which is 30–100 cm long and is characterized by its strong odor (Kriter Basu, 1975).

Indian and Eastern Asian people use spearmint as a common constituent in their diet. It is used with spices to give the food a special flavor and fragrance, also used for flavoring chewing gums, toothpaste, confectionery and pharmaceutical preparations [Sugimura *et al*2004].Spearmint essential oil is a common constituent in hygiene and cosmetic products, and substantial amounts are used in the food and beverage industries [Bhattacharya *et al*1999]. The dry or fresh leaves of spearmint are added by the Middle East and African during the brewing of tea, where it provides a pleasant aroma and refreshing taste [Biswas, 2011]. There was an investigation that confirmed that spearmint had significant inhibitory effects against the cooked meat heterocyclic amine mutagen both *in vitro* and *in vivo Mentha spicata* has high traditional medicinal value as it is one of the important constituents of Ayurveda, Homeopathy and Siddha systems of medicine. *Menthal* can be used for common cold, cough, sinusitis, fever, bronchitis, nausea, vomiting, indigestion, intestinal colic and loss of appetite. It can have a calming effect when used for insomnia or massages. Essential oil of Spearmint was found to have some antimicrobial activity. It is also a safe and effective therapeutic option for the treatment of chemotherapy induced nausea and emesis in patients.

PUNICA GRANATUM

Punicagrantanum L,commenly known as pomengrate family *puniaceae* belong to family *Lythraceae* . The leaves are shiny and about 7.6 cm long (Hedge Chaitra *et al*.,2012) .

In the ancient Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years. The rind of the fruit and the bark of the pomegranate tree is used as a traditional remedy against diarrhea, dysentery and intestinal parasites. The seeds and juice are considered a tonic for the heart, throat, eyes and for a variety of purposes, such as stopping nose bleeds and gum bleeds, toning skin, firming-up sagging breasts and treating hemorrhoids. (Chithra2000)

In the past decade, numerous studies on the antioxidant, anti carcinogenic, and anti-inflammatory properties of pomegranate constituents have been published, focusing on treatment and prevention of cancer, cardiovascular

disease, diabetes, dental conditions, erectile dysfunction, bacterial infections and antibiotic resistance, and ultraviolet radiation-induced skin damage. Other potential applications include infant brain ischemia, male infertility, Alzheimer's disease, arthritis, and obesity. In treating diarrhea, dysentery and intestinal parasites. Pomegranate is well known for antioxidant properties. It helps in preventing the formation of skin cancer by reducing the frequency of lesions. It provides relief from minor skin irritations, such as dry skin, eczema and psoriasis. (Jain, 2000)

Health Benefits of Pomegranate

Medicinal Benefits

Pomegranate is a poly-vitamin, a unique fruit plant producing a wide spectrum of biologically active substances especially important in our present-day polluted environment. It helps in preventing the harmful effects of radioactive substances by producing biologically active substances. Russians, after the deadly Chernobyl tragedy, used pomegranates to reduce the effect of radioactive substances. In order to maintain the health and energy levels of astronomers, submariners and coal miners, they often consume pomegranate juice regularly. (Mohammad Ali Ebrahimzadeh *et al.*, 2008) Pomegranate is loaded with tannins, anthocyanin, and polyphenols' and antioxidant vitamins, A, E and C, all of which have a health effect on the body. These elements work together to benefit the arteries, plus it keeps the cardiovascular system healthy which is the chief health benefit of Pomegranate It has also been found to increase levels of nitric oxide, which improve blood flow to the heart, reduce arterial plaque, reduce systolic blood pressure and help in curing erectile dysfunction. Other benefits include preventing premature aging, stroke, arthritis, Alzheimer's and even cancer. The juice of the red pomegranate has received attention for its rich flavor and health boosting properties. The juice comes from the crushed seeds. Pomegranate juice has been shown to contain more antioxidants than most fruit juices, red wine or green tea, according to Health Castle. (Kulkarni *et al*.,2001)

SOLANUM NIGRUM

Solanum nigrum (black nightshade) is a medicinal plant member of the *Solanaceae* family of plants. !is family comprises many genera, well known for their therapeutic properties. In addition to *S. nigrum*, this family includes fruits and vegetables such as potato (*Solanumtuberosum*), tomato, and peppers, ornamental plants such as petunia, and other medicinal plants such as *Atropabelladonna* L. (deadly nightshade), *Daturastramonium* L. (Jimson weed), and *Hyoscyamusniger* L. (black henbane). *S. nigrum* has been extensively used traditionally to treat various ailmentssuch as pain, inflammation and fever. plant is also used in the Orientalsystems of medicine for various purposes – as a antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic agent. Various compounds have been identified which are responsible for diverse activities. (Jain *et al* 2011)

S. nigrum has been used traditionally to treat various ailments such as pain, inflammation fever5 and enteric diseases. It possess many activities like antitumorigenic, antioxidant, anti-inflammatory, hepatoprotective, diuretic, and antipyretic agent, antibacterial, mycotic infection, cytotoxicity, anti-convulsant, antiulcerogenic. It is also used against sexually transmitted diseases. Ethanol extract of *Solanum nigrum* is used to determine phytochemicals contents of leaves of *Solanumnigrum* L. Subjected to different processing methods were evaluated Processing procedures adopted include shredding, sun–drying, oven–drying, steaming and a combination of these. Minerals examined are Na, K, Ca, Mg, Fe, P, and Zn while the phytochemicals are alkaloids, flavonoids, hydrocyanic acid, phenols, phytic acid and tannins. Oven – drying was the most effective method for retaining the studied minerals in *S. nigrum* but only for Na, Ca, Fe and Mg. The phytochemical screening of the crude extract show the presence of alkaloids, reducing sugars, tannis, flavonoids, phlobatannins and steroids. (Zakaria *et al*.2006)

The present study is focus on the phytochemical analysis, Antioxidants and Antimicrobial activity

of medicinal plant against fish pathogens. Such *as E .coli , Staphylococcus aureus, Klebsilla, Salmonella , Streptococcus* . Finally plant extracts used we analyses for minimal inhibitory concentration by fish pathogens and agar diffusion method. Thin layer chromatographyare used. TLC Plates are used for bio autography we can identify the phytochemical constituents and screening the antimicrobial activity of the plant extracts.

MATERIALS AND METHODS

Plant collection

Plants leaves were collected from home. Leaves of this plant were dried at 25◦C and then grounded with a blender (Coffee grinder Model A979) and stored at approximately 4C until required for use.

Preparation of Plant Extract

10 g of powdered leaves were placed in conical flask and 100 ml of methanol was added and plugged with cotton. The powder material was extracted with methanol for 24 hours at room Temperature with continuous stirring. After 24 hours the supernatant was collected by filtration and the solvent was evaporated to make the crude extract. The residues obtained were Stored in airtight bottles in a refrigerator for further use.

QUALITATIVE DETERMINATION

Preliminary Phytochemical Screening

The methanol extracts of following plants was subjected to different chemical tests for the detection of different phyto constituents using standard procedures.

Test for Tannins:

1 ml of the sample was taken in a test tube and then 1 ml of 0.008 M Potassium ferricyanide was added. 1 ml of 0.02 M Ferric chloride containing 0.1 N Hydrochloric acid was added and Observed for blue-black colouration.

Test for Phlobotanin

When crude extract of each plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

Test for Saponins:

Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. Add some drops of olive oil. The formation of stable foam was taken as an indication for the presence of saponins.

Test for Flavonoids:

5 ml of dilute ammonia solution were added to a portion of the crude extract followed by addition of concentrated H2SO4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.

Test for Alkaloids:

Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

Test for Quinones:

Dilute NaOH was added to the 1 ml of crude extract. Blue green or red coloration indicates the presence of quinones.

Test for Coumerin:

10 % Sodiumhydroxide (NaOH)was added to the extract and chloroform was added for observation of yellow color, which shows the presence of caumarrins.

Test for Terpenoids (Salkowski test):

5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H2S04 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

QUANTITATIVE DETERMINATION

Determination of Total Phenolic Content

Total Phenolic Content (TPC) in entophytic extracts was determined using Folin-Ciocalteu's colorimetric method. To 5 ml of 0.3% HCl in methanol/deionized water (60:40, v/v), 100 mg of the ethanol extract was added. From the resulting mixture (100 µl) was added to 2 ml of 2% aq-ueous sodium carbonate. The mixture was incubated for 2 min. To that 100 µl of 50% Folin-Ciocalteu's reagent was added and incubated for 30 min, absorbance was measured at 750 nm against blank. The content of total phenol was

calculated on the basis of the calibration curve of Gallic acid and the results were expressed as mg of Gallic acid equivalents (GAEs) per g of extract].

Flavonoid Determination

The leaves extract (250 µl) was mixed with distilled water (1.25 ml) and NaNO2 solution (5%, 75 µl). After 5 min theAlCl3 H2O solution (10%, 150 µl) was added. After 6 min, NaOH (1M, 500 µl) and distilled water (275 µl) were added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm against blank. The content of flavonoid was calculated on the basis of the calibration curve of quercetin and the results were expressed as mg of quercetin equivalents per g of extract

Total ascorbic acid

Total ascorbic acid was determined by CUPRAC assay in 100 mg plant sample and 5 ml of water .The absorbance of formed bis (NC) copper (i) chelate was measured at 450 nm.

Total tannin content

The plant extracts are taken in 1 ml and 4% 2 ml methanol was added and measuring at 500 nm

Antioxidant Activities of the Extracts

DPPH Radical Assay (1,1-diphenyl-2-picryl-hydrazyl)

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple coloured methanol solution of DPPH. This spectrophotometric assay uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Gulluce *et al*., 2006). Fifty micro liters of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against s blank at 517 nm. Inhibition free radical DPPH in percent (I%) was calculated in the following way:

I%= A blank- (A sample/A blank)x100,

Where A blank is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound. Extract concentration form the graph plotted inhibition (IC50) was calculated from the graph plotted against extract concentration. Synthetic antioxidant reagent butylatedhyroxytoluene (BHT) was used in triplicate.

Ferric Thiocyanate (FTC) Method (Govindhappan *et al* 2000)

Different extracts (4 mg) and standards (4 mg; BHT, vitamin C and vitamin E) were mixed with 4 ml of absolute ethanol, 4.1 ml of 2.52% linoleic acid in absolute ethanol, 8 ml of 0.02 M phosphate buffer (pH 7.0) and 3.9 ml of distilled water. The mixture was placed at 40°C (0.1 ml) and was then mixed with 9.7 ml of 75% (v/v) ethanol and 0.1 ml 30% ammonium thiocyanate. Three minutes after adding ferrous chloride (0.1 ml of 2 × 10−2 M ferrous chloride), the absorbance was measured at 500 nm in a spectrophotometer. This step was repeated every 24 h until the control reached its maximal absorbance value. The mixture without added sample was used as a control [24]. The inhibition of lipid peroxidation (%) was estimated by the following formula:

%= Inhibition100A1/A0 x100

where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts .

Thiobarbituric Acid (TBA) Method (Govindhappan *et al* 2000)

Plant Extracts (2 ml) and standard solutions (2 ml) on the final day (day 8) of the FTC assay were added to 1 ml of 20% aqueous Trichloroacetic acid and 2 ml of 0.67% aqueous Thiobarbituric acid. After boiling for 10 min, the samples were cooled. The tubes were centrifuged at 3000 rpm for 30 min. Absorbance of the supernatant was evaluated at 532 nm in a spectrophotometer. The antioxidant activity was calculated by percentage of inhibition in this method as follows:

%=Inhibition100A1/A0x100

Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts.

Free Radical Scavenging Activity

The ability of methanol and chloroform extracts of plants to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radicals was estimated . MC extracts (3 ml) with six different concentrations (15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) were mixed

with 1 ml of a 0.1 mM methanol solution of DPPH. The absorbance was measured by a spectrophotometer at 517 nm at 30 min intervals against a blank (pure ethanol). The percentage of radical scavenging activity was calculated using the following formula.

Radical scavenging%=1(A0-A1/A0x100)

Where A0 is the absorbance of the control and A1 is the absorbance of the sample extracts. Lower absorbance values show higher free radical scavenging activity. Ascorbic acid was used as a reference standard in different concentrations (1.56, 3.12, 6.25, 12.5, 25 and 50 µg/ml). The 50% inhibitory concentration value (IC50) is indicated as the effective concentration of the sample that is required to scavenge 50% of the DPPH free radicals.

Iron Chelating Activity

The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. The reaction mixture containing 1 ml of 0.05% O-Phenanthroline in methanol, 2 ml ferric chloride (200 µM) and 2 ml of various concentrations ranging from 10 to 1000 µg was incubated at room temperature for 10 min and the absorbance of the same wasmeasured at 510 nm. EDTA was used as a classical metal chelator . The experiment was performed in triplicates.

Reducing Power Method (Kumaran et al., 2007)

1m1 of plant sample was mixed with 2.5 m1 phosphate buffer (0.2 M) pH 6.6 and 2.5 m1 (1%) (K3(Fe(CN}t;); the mixture was incubated at room temperature for 20 minutes , after which 2.5 ml (10%) trichloroacetic acid was added. The whole mixture was then centrifuged (650 x *g* at room temperature) for 10 minutes, 2.5 m1 upper layer was removed and 2.5 ml H20 and 0.5 ml (0.1%) Fecl2 were added. Triplicates solutions were prepared. This was allowed to stand for 30minutes the absorbance was measured at 700 nm.

Thin layer chromatography (TLC)› phytochemical assay

The plant extracts were spotted onto a silica gel TLC plate (Kieselogel 60 F254 0.2 mm, Merck). The plates were developed in ascending direction with toluene: acetone: methanol (1:1:3) and CHCl3: acetone: methanol: H2O (1:7:4:5) as mobile phase. Spots were visualized by UV irradiation at 366 nm after spraying with AlCl3 reagent (Harbone, 1973; Wagner et al., 1984) for phenolic/ flavonoid compounds, acidic iodine-potassium iodide for alkaloids, perchloric acid for sterols (Hara, 1963), Benedicts reagent for caumarrins and flavonoids, vanillin-HCl reagent for proanthocyanidins (Ribera- Gayon, 1972), and antimony (III) chloride reagent for cardiac glycoside (Wagner et al., 1984).

ISOLATION AND SCREENING OF FISH PATHOGENS

Fish samples *viz*., matthisankara, prawn collected from the erode fish market. Collected samples were aseptically taken to the Biotechnology lab for bacteriological examination. The chosen fish were cut down into small pieces, sterilized/washed with 70% absolute ethanol and later d3H2O (double distilled deionized water) for 5 min to remove excess ethanol. Washed sample products were placed on Nutrient agar (NA) medium supplemented with methyl red and crystal violet. After the incubation of 24 hours at 37ºC, the small portion of growth area were picked with sterilized loop and again streaked on the different selected medium such as Nutrient gar (NA; supplemented with crystal violet and methyl red), Macconkey agar (MA), Eosin methyleneblue (EMB) ,Blood agar(BA) , Bismuth sulphate agar(BSA), Neutral red chalk lactose agar(NRCLA) and also biochemical tests respectively for the screening of single pathogen. Stock cultures were grown in Nutrient Broth at 37ºC and stored at -20ºC as 60% glycerol stock before used for Antibacterial analysis.

Biochemical Tests of Isolated Pathogens (microbiology Pelzer)

The gram staining was aimed at differentiating gram reactions, sizes, shapes and arrangement of cells of the isolates. Various biochemical tests such as oxidase, catalase, urease, H2S, citrate utilization, Voguesproskeur test were used for the confirmation of test pathogen.

Sensitivity Test of Antibiotics

Sensitivity of antibiotics against test strains was determined by filter disc diffusion method

Sensitivity was predictable with clear zone surrounding the disc. The potency of antibiotics (5 mm in diameter) per disc are as follows; Amoxicillin (10 µg), Streptomycin (10 µg), Tobramycin (10 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Sulfomethoxyzol (25 µg), Tetracycline (10 µg), Penicillin G (10 µg), Trimethoprim (5 µg), Ampicillin (10 µg).

Antibacterial Activity through Filter Disk Diffusion Method

Filter disc diffusion method was used for testing of medicinal plant extracts against four bacterial fish pathogens viz., *Escherichia coli, Klebsilla, Streptocoocus, Salmonella, Staphylococcusaureus* , Whatman No. 1 filter paper disc (5 mmdiameter) was impregnated with crude (10 µl) plant extracts was placed on Muller Hinton agar. This was previously swabbed with bacterial fish pathogens. The sterile discimpregnated with only solvents used as a negative control. All the plates were incubated at37ºC for 24 hours under static conditions. After 24 hours the zone of inhibition appearing aroundthe discs were measured and recorded in millimeter (mm) diameter. Each experiment wasconducted thrice, and the mean of the results were calculated for both the test and control.

Preparation of Extract and Antibacterial Activity

The plant leaves were collected and dried. The sample was spread out during daylight hours for 5 days until it dried to brittleness. Dried peels were powdered to get 60- mesh size using a mixer grinder. Thirty grams of powdered peel was extracted with different solvents like Hexane, ethanol and methanol with occasional shaking for 3 days at room temperature. The extracts were filtered, concentrated and dried at 50oC and the weight of each residue was recorded. (Shiv Shanker Gautam *et al*., 2011)

Minimum Bactericidal Concentration

The MBC was performed to test the antimicrobial activity of active extract using tube dilution method. The MBC was defined as lowest concentration able to kill any microbe. Dilutions of the plant extract were prepared in sterile nutrient broth to get a final concentration of 2 mg, 4 mg, 8 mg, 16 mg, 32 mg and 64 mg/ml respectively (Mathur*et al*., 1996). To each of these dilutions, a loop full of STEC culture adjusted to 0.5 Mac Farlandstandards, was inoculated and all the tubes were incubated at 37oC for 24 hrs. After incubation, loopfull from each tube was inoculated onto nutrient agar plates. The plate without growth was recorded as MBC.

Preparation of agar wells for different solvent extracts

5 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 50 µl solvent extract was transferred by micropipette per well. Plates were Immediately kept at 4oC in refrigerator for 1 h for the diffusion of extract.And then shifted to 35oC or .5oC in incubator. Zone of inhibition was measured after 24 h of incubation.For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The control zones were subtracted from the test zones and the resulting zone diameter is obtained.

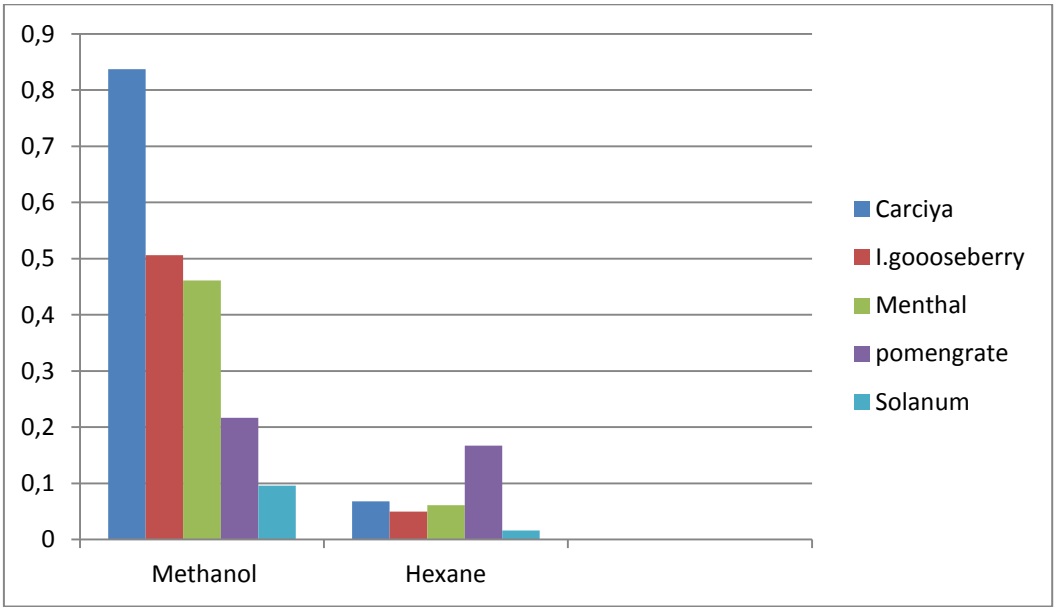
Thin Layer Chromatography and TLC Bio autography Analysis

The methanol extracts were applied at 2.5 cm from the base of silica gel G TLC plate. The TLC plates were developed with ethyl acetate: methanol: water (81:11:8) and were run in duplicate. 5% ferric chloride reagent was sprayed and the chromatogram was observed. The second set of chromatogram, developed as described above in TLC plates. Which was not subjected to chemical treatment for observing spots was loaded with the inoculum of test organisms in molten Mueller Hinton Agar, over the TLC plates. After the solidification of the medium, the TLC plate

was incubated overnight at 37oC. Subsequently, bioautogram developed was sprayed with 1% aqueous solution of 2, 3, 5, triphenyl tetrazolium chloride (TTC) and incubated at 37C for 4 h. Inhibition zone indicated the presence of active compounds (Anjana*et al.*, 2010). Ellagic acid and the antibiotic rifampin were used as controls.

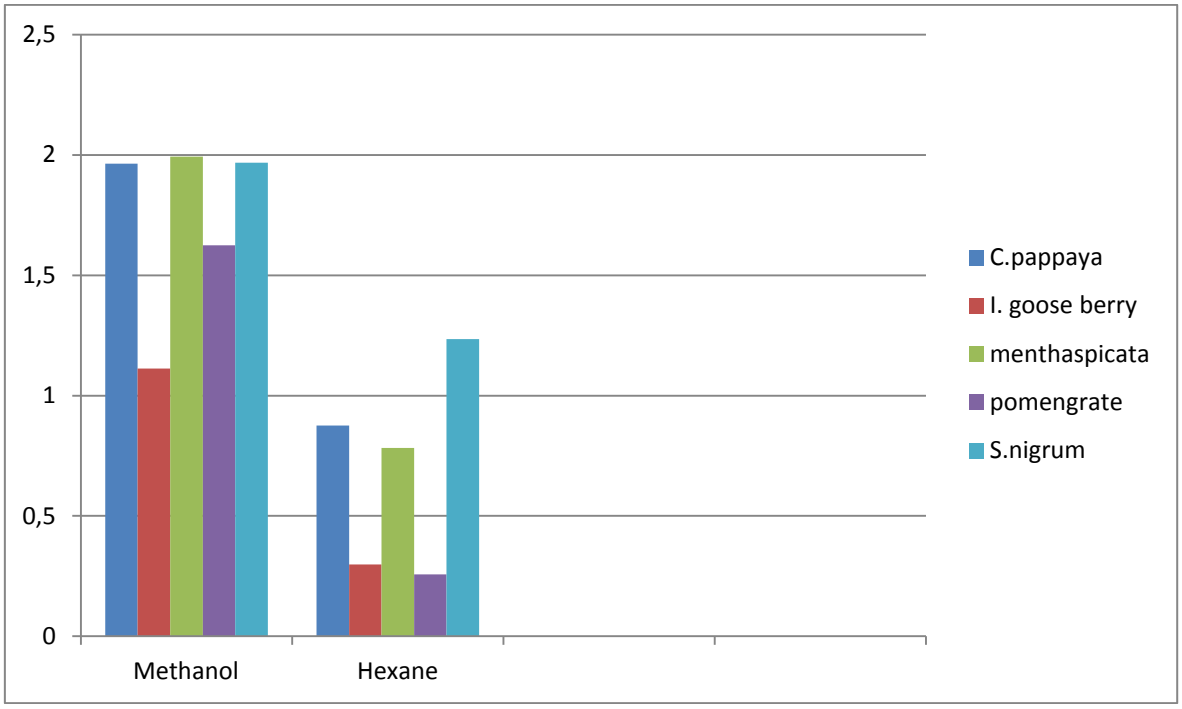
TOTAL ASCORBIC ACIDTABLE 2

Plants extracts (m)	**OD At 450 nm**	**Capacity**	**Plant extracts (H)**	**OD At 450 nm**	**Capacity**
Carciya papaya		**0.837**	*Carciya papaya*		**0.868**
Indian gooseberry		**0.506**	*Indian gooseberry*		**0.050**
Menthaspicata		**0.461**	*Menthaspicata*		**0.061**
pomengrate		**0.217**	*pomengrate*		**0.167**
Solanum nigrum		**0.096**	*Solanum nigrum*		**0.016**



TOTAL TANIN CONTENT TABLE 3

Plant extracts	**OD at 500nm**	***Carciya papaya***	***Indian gooseberry***	***Mentha spicata***	***pomengrate***	***Solanumnigrum***
Methanol		**1.963**	**1.113**	**1.993**	**1.625**	**1.967**
Hexane		**0.876**	**0.298**	**0.783**	**0.257**	**0.235**



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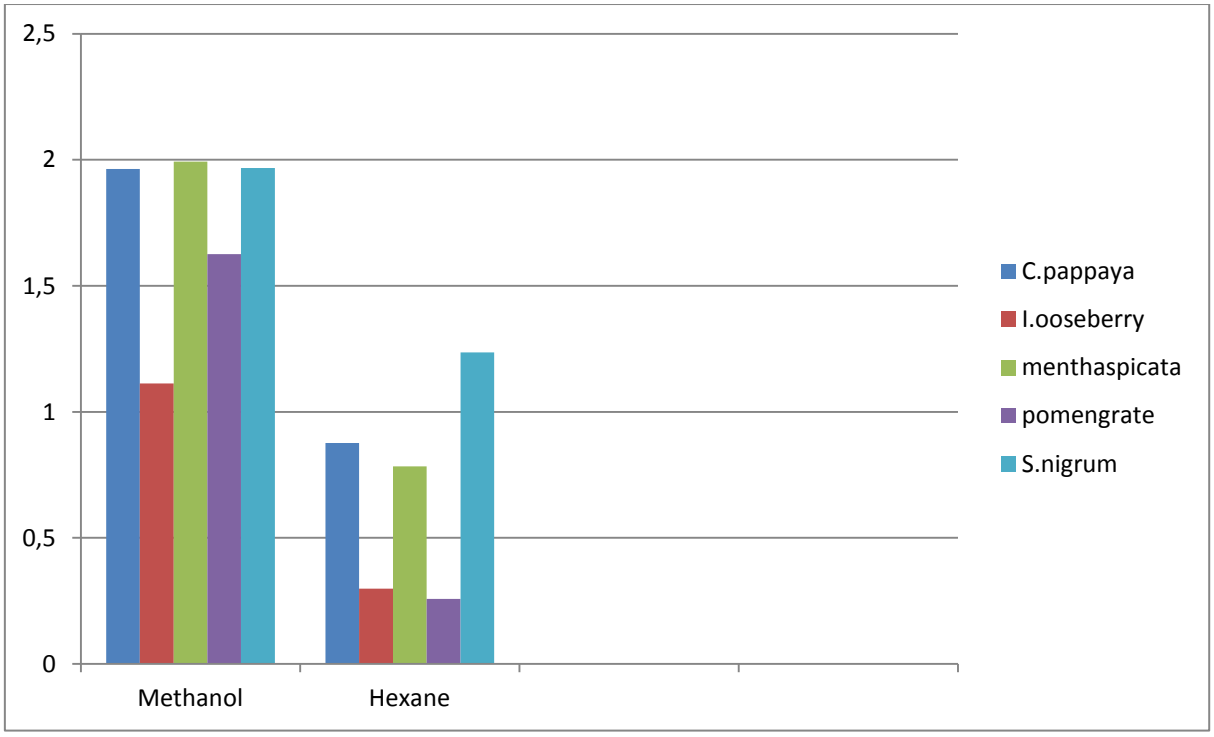
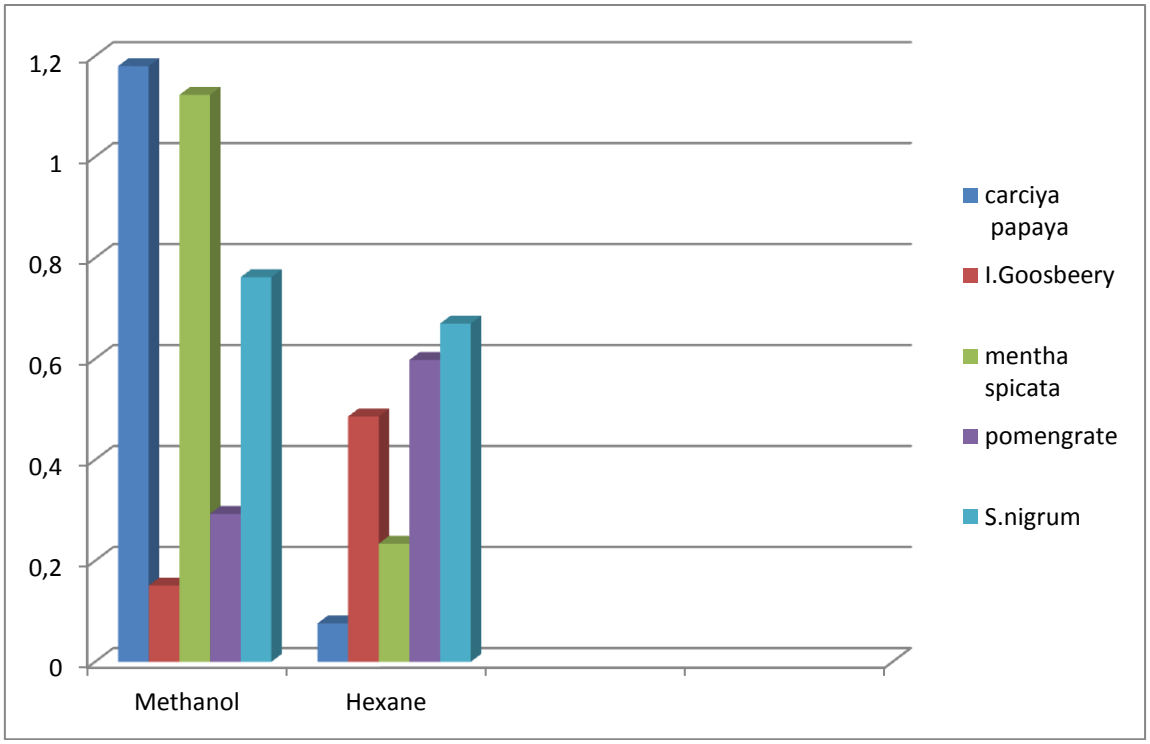
Methanol		**1.963**	**1.113**	**1.993**	**1.625**	**1.967**
Hexane		**0.876**	**0.298**	**0.783**	**0.257**	**0.235**



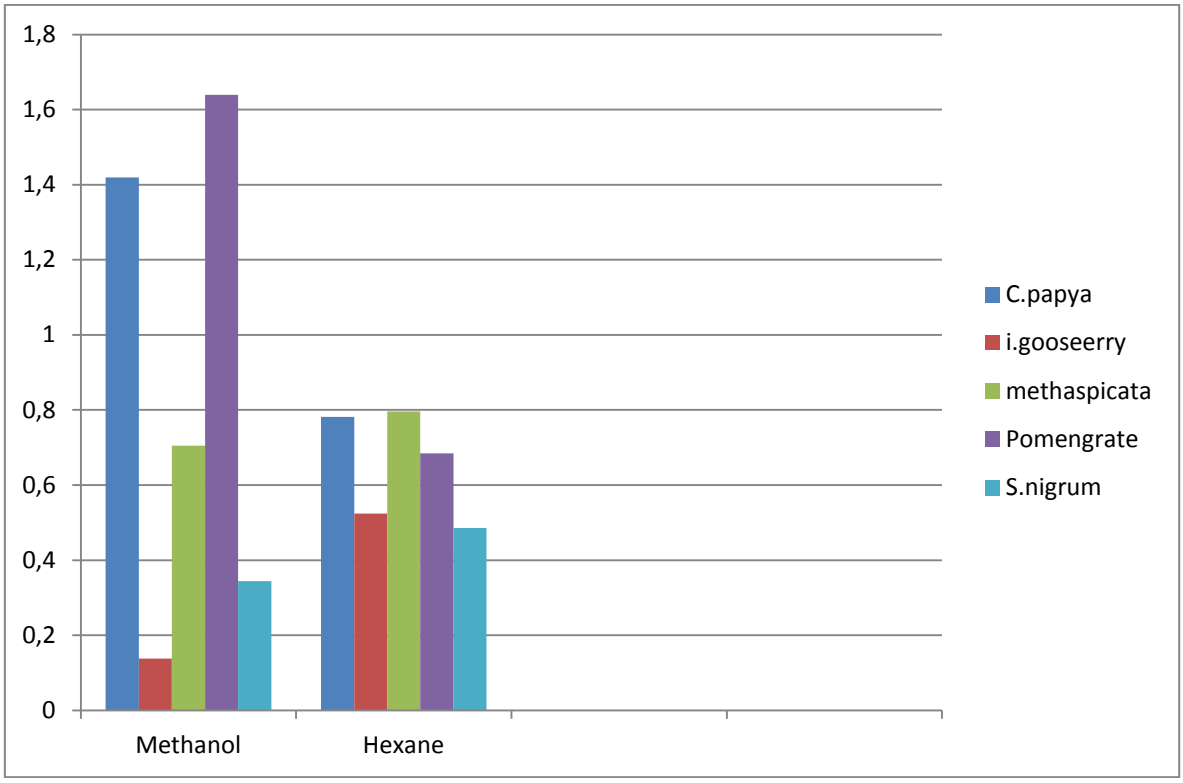
TOTAL PHENOL CONCENTRATION TABLE 4

Plant extracts	ODat 760nm	*Carciya papaya*	*Indian gooseberry*	*Mentha spicata*	*pomengrate*	*Solanum nigrum*
Methanol		**1.180**	**0.151**	**1.123**	**0.293**	**0.762**
Hexane		**0.076**	**0.486**	**0.234**	**0.598**	**0.670**



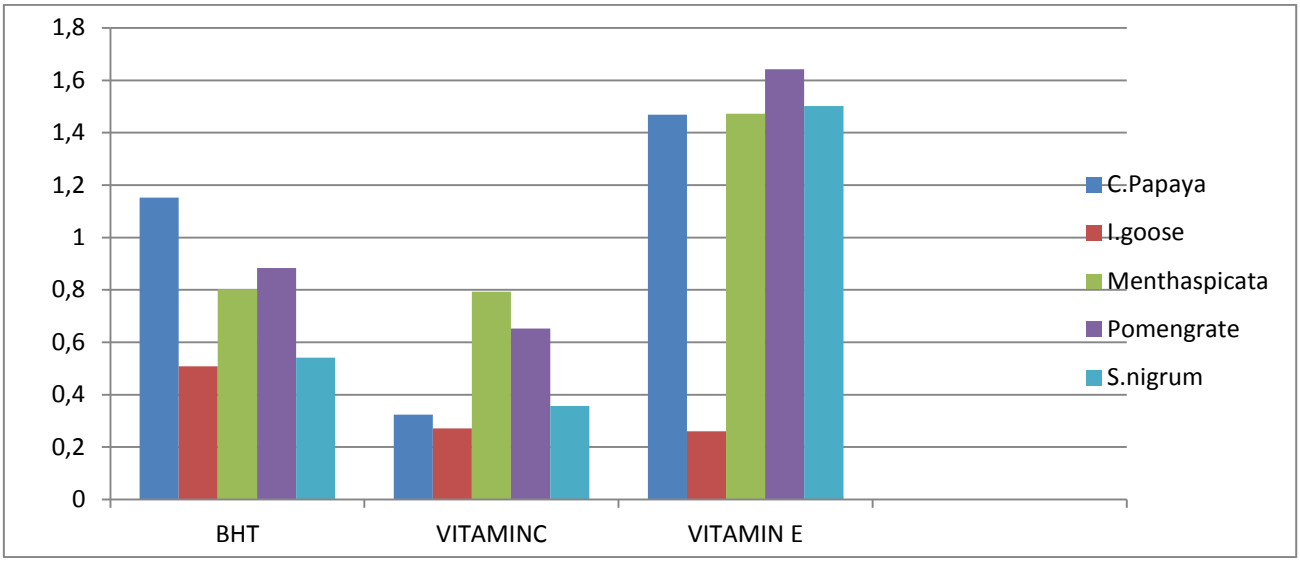
TOTAL FLAVANOID DETERMINATION TABLE 5

Plant extracts	*C.papaya*	*Indian gooseberry*	*Menthaspicata*	*pomengrate*	*Solanum nigrum*
Methanol	**1.419**	**0.138**	**0.702**	**1.639**	**0.344**
hexane	**0.782**	**0.524**	**0.796**	**0.684**	**0.486**



TOTAL ANTIOXIDANT CAPACITY TABLE6

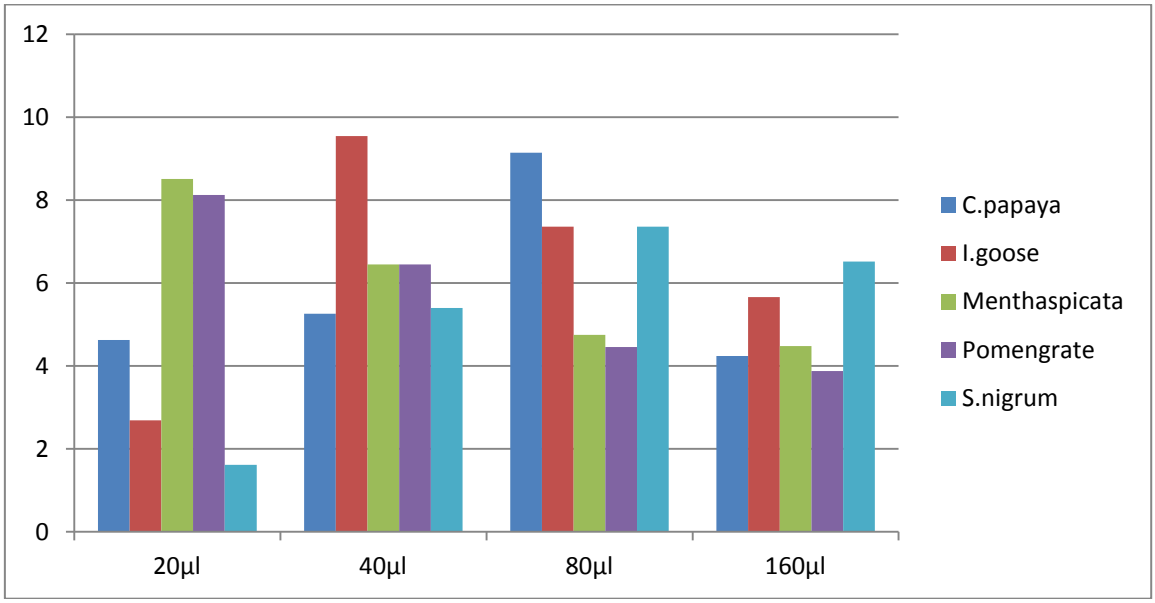
Plant extracts(m)	OD at 595 nm BHT	%of inhibition 100A1xA0/100	Vitamin C OD at 595 nm	%of inhibition 100A1xA0/100	Vitamin E OD at 595 nm	%of inhibition 100A1xA0/100
C.papaya	1.973	1.152	0.319	0.324	1.734	1.469
I.gooseberry	0.871	0.508	0.267	0.2711	0.311	0.260
Mentha spicata	1.37	0.8025	0.721	0.7936	1.739	1.473
Pomengrate	1.513	0.8837	0.643	0.653	1.938	1.642
S.nigrum	1.03	0.541	0.351	0.3567	1.773	1.502



% inhibition of total antioxidant

HYDROXYL RADICAL SCAVENGING ACTIVITY TABLE 7

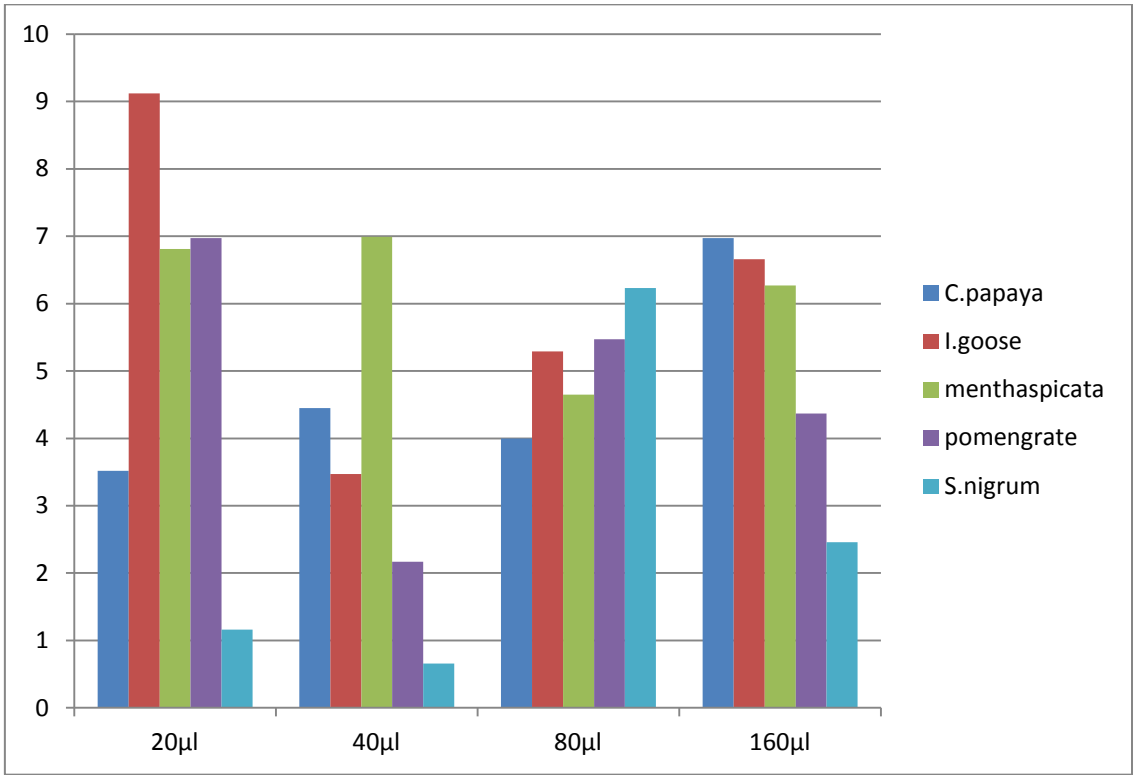
Extracts	*C.papaya*	*I.gooseberry*	*Menthaspicata*	*pomengrate*	*Solanumnigrum*
20µl	4.63	2.69	8.51	8.12	1.62
40µl	5.26	9.54	6.45± 0.01	6.45	5.40
80µl	9.14	7.36	4.75	4.46	7.36
160µl	4.24	5.66	4.48	3.88	6.52



% Inhibition hydroxyl radical scavenging

ANTIOXIDANT ACTIVITY TABLE 8

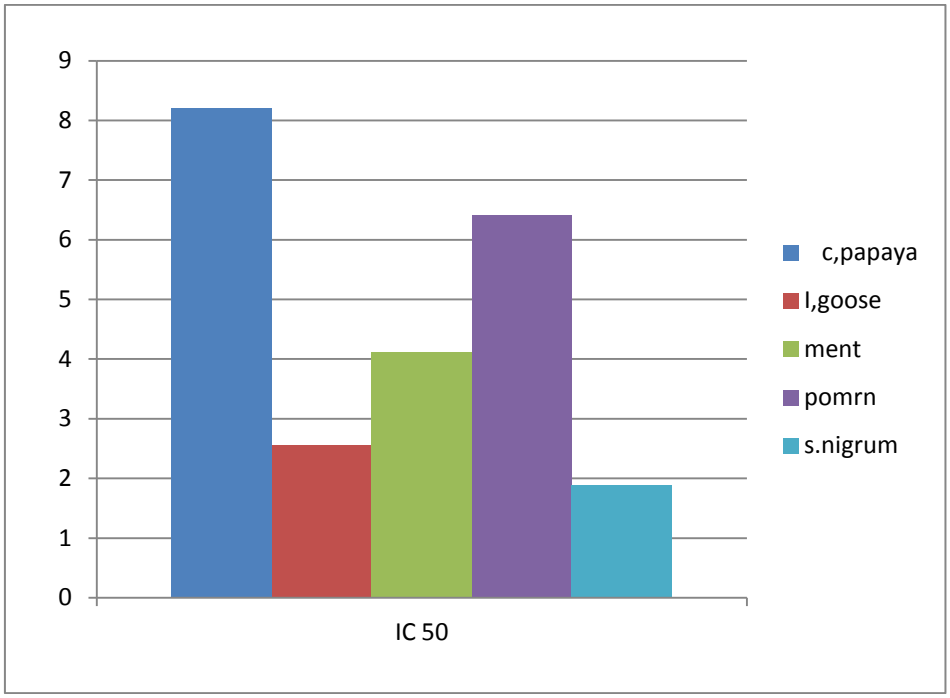
Extracts	*C.papaya*	*Indian gooseberry*	*Menthaspicata*	*pomengrate*	*Solanumnigrum*
20µl	**3.52**	**9.12**	**6.81**	**6.97**	**1.16**
40µl	**4.45**	**3.47**	**6.99**	**2.17**	**0.66**
80µl	**4.0.**	**5.29**	**4.65**	**5.47**	**6.23**
160µl	**6.97**	**6.66**	**6.27**	**4.37**	**2.46**



% inhibition of DPPH radical

IORN CHELATING ACTIVITY TABLE 9

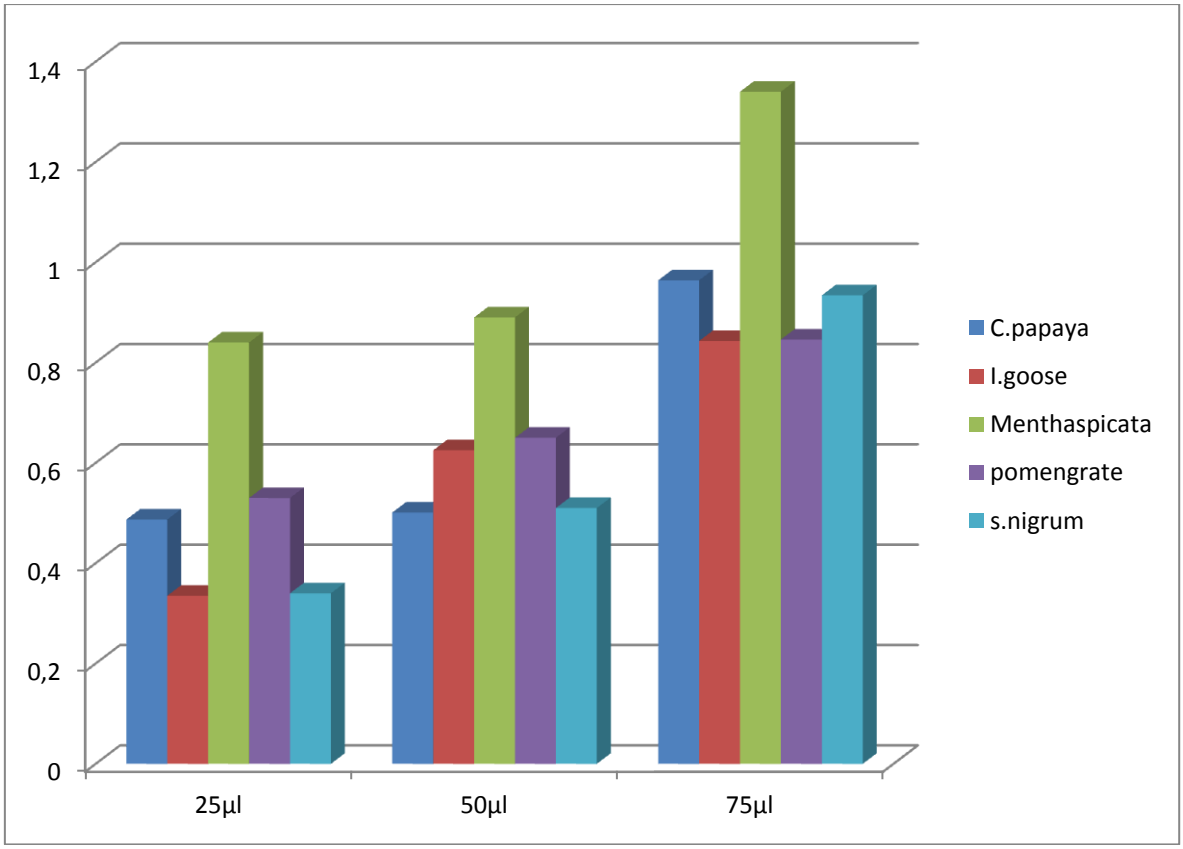
Name of the plant	Fe2+ chelating activity (IC50 mg/ml)
carciya papaya	8.2
I.gooseberry	2.50 ± 0.01
Menthaspicata	4.11 ± 0.01
Pomengrate	6.40 ± 0.02
Solanum nigrum	1.83 ± 0.16



% inhibition of Iorn chelating activity

REDUCING POWER ASSAY TABLE 10

Plant extracts/µl	***Carciya papaya***	***I.gooseberry***	***Menthaspicata***	***Pomengrate***	***S.nigrum***
25 µl	0.487	0.335	0.84	0.53	0.34
50 µl	0.501	0.625	0.89	0.65	0.51
75 µl	0.964	0.843	1.34	0.846	0.934



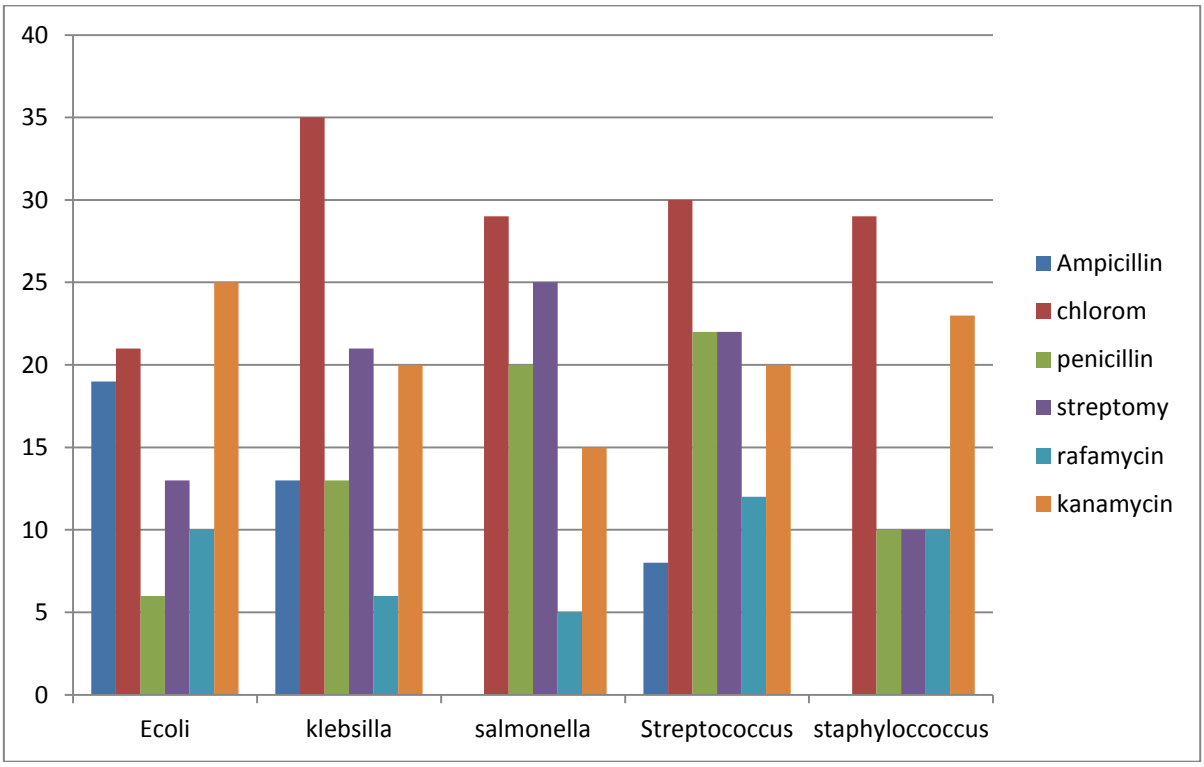
BIOCHEMICAL TEST/CARBOHYDRATE FERMENTATION TEST /DIFFERENTIAL MEDIA
TABLE 11

S.NO	Biochemical /differential media	MATTHI A May be E.coli	MATTHI B	PRAWN A May be Klebsilla	PRAWN B May be salmonella	SANKARA A May be saphylococcus	SANKARA B May be streptococcus
1.	Indole	P+	P+	N-	N-	N-	N-
2.	MR	P+	P+	N-	P+	P+	P+
3.	VP	N-	N-	N-	N-	P+	N-
4	CITRATE	N-	N-	P+	N-	N-	P+
.5.	GLUCOSE	P+	P+	P+	N-	N-	ACID/P+
6.	LACTOSE	P+	P+	P+	N-	N-	ACID/P+
7.	MANITOL	N-	P+	P+	P+	A/GAS	ACID/P+
8.	SUCROSE	P+	N-	P+	P+	A/NO GAS	ACID/P+
9.	CATALASE	P+	N-	P+-	P+	P+	N-
10	OXIDASE	N-	N-	N-	N-	P+	N-
11.	NITRATE	P+	P+	P+	N-	P+	N-
12	H2S	N-	N-	N-	N-	N-	N-
13	UREASE	P+	P+	N-	N-	N-	N-
.14	TSI	A/A	A/A	A/G	A/A	A/A	AL/AL
15.	STARCH	N-	P+	P+	N-	N-	N-
16	CASEIN	N-	N-	P+	N	N-	N-
17	SIM AGAR	N-	N-	N-	P+	P+	P+
18	DIFFERENTIAL MEDIA						
19	EMB	Greenic metallic sheen colony	Greenic metallic sheen colony	Pink colour colony	Colour less colony	Pink colours colony	Pink colour colony
20	MACCONKEY	Pink colourless colony	Pink colourless colony	Colourless colony	Pink colourless colony	Pink colour colony	Pink Colour colony
21	BLOOD AGAR	no	no	no	no	Bete hemolysis occur	Alpha hemolysis occur
22	MANNITOL SALT	no	no	no	colour less colony	Pink colour colony	Golden colour colony
23	BISMUTH	no	no	no	Black colour colony	no	Black colour colony
24	NEUTRAL RED	Pink colour colony	Pink colour colony	Light pink colour colony	no	pink colour colony	pink colour colony

A/A-Acid butt alkaline slant A/G- Alkaline butt gas production N- Negative P+ Positive

ANTIBIOTIC SENSITIVITY TEST TABLE12

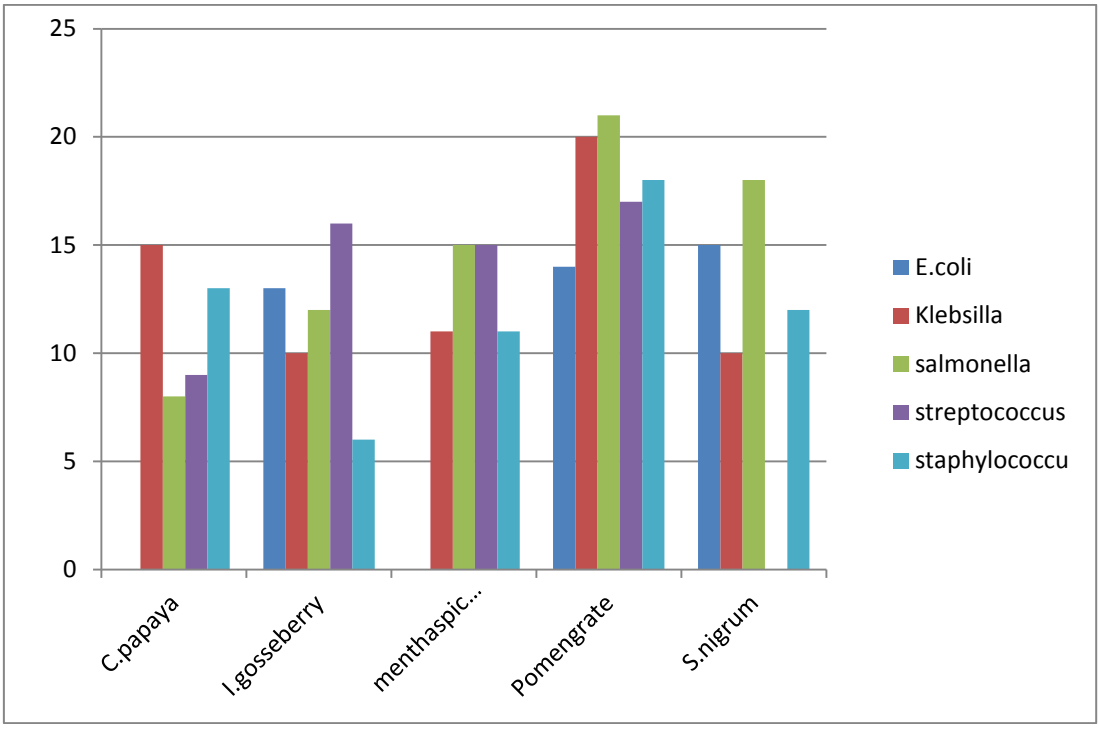
Organisms	Ampicillin	chloromphenicol	pencillin	streptomycin	rafomycin	kanamycin
E.coli	**18±1**	**21±2**	**6±1**	**10±3**	**10±1**	**25±1**
Klebsilla	**12±1**	**35±1**	**13±1**	**20±1**	**6±1**	**20±1**
Salmonella	**No**	**28±1**	**20±1**	**25±1**	**5±1**	**15±1**
Streptococcus	**8±1**	**30±2**	**20±2**	**20±2**	**12±1**	**20±1**
staphylococcus	**No**	**29±1**	**10±3**	**10±2**	**10±1**	**23±2**



% inhibition of microorganisms

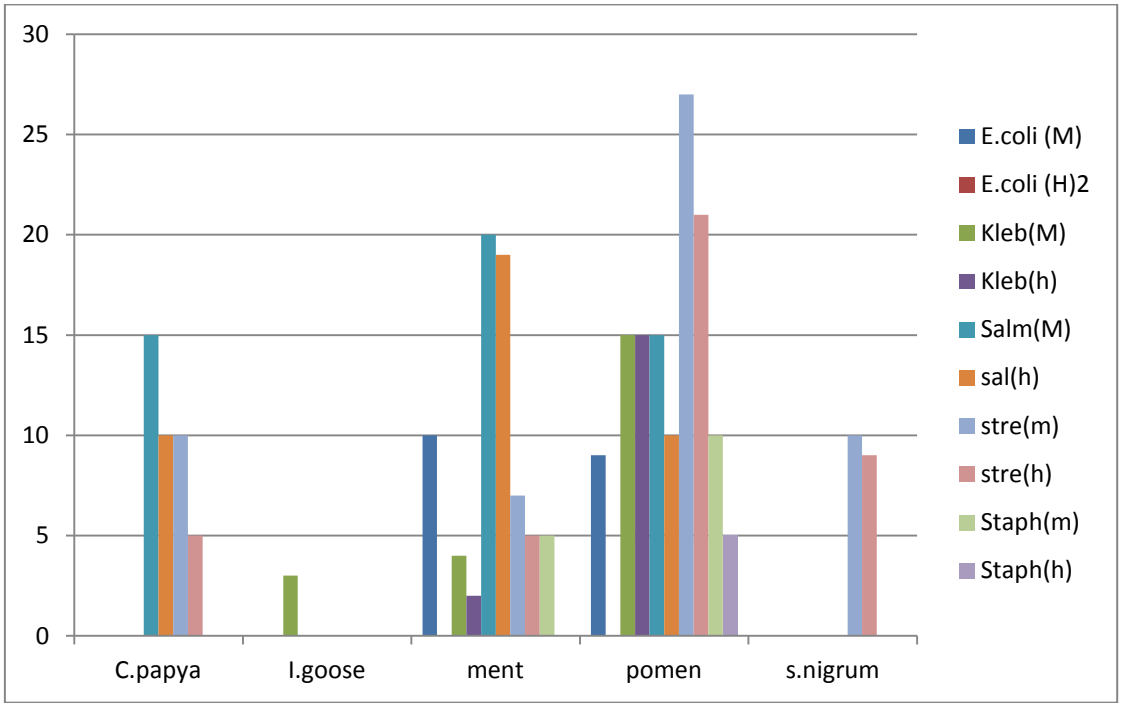
ANTIBACTERIAL ACTIVITY THROUGH DISK DIFFUSION METHOD TABLE 13

Plant extraction	***E.coli***	***klebsilla***	***salmonella***	***streptococcus***	***Staphylococcus***
C.papaya	No	15±1	8±3	9	13
I.gooseberry	13	10	12	16	6±3
Menthaspicata	No	11	15	15±1	11
Pomengrate	14	20	21	17	18
s.nigrum	15±1	10	18	No	12±1



PREPERATION OF AGAR WELLS FOR DIFFERENT SOLVENT EXTRACTION TABLE 14

Plant extraction	***E.coli***		***Klebsilla***		***Salmonella***		***Streptococcus***		***Staphylococcus***	
	M	***H***	***M***	***H***	***M***	***H***	***M***	***H***	***M***	***H***
C.papaya	No	No	No	No	15±1	10	10±1	5±2	No	No
I.gooseberry	No	No	3±1	No	No	No	No	No	No	No
Menthaspicata	10±2	7±3	4±1	2±1	20±1	19±2	6±1	5±2	5±1	No
Pomengrate	9±3	No	13±2	15±1	15±1	10	27±3	21±1	10±1	5±1
s.nigrum	No	No	No	No	No	No	10±2	9±**3**	No	No



H-HEXANE M-METHANOL

SUMMARY AND CONCLUSION

- ✓ . The plants are having lot of medicinal properties and we can use in normal life. It is extensively used in Ayurveda medicine and folk medicine .
- ✓ We can use home plants such as *pomengrate (Punica-granatum), Indian goose berry(alma) ,Solanum nigrum, Mentha spicata (Menthol), Carciya papaya(papaya)* The present study to investigate and screening the phytochemical contents from the plant extracts .
- ✓ All the five plants are had flavonoid, tannin phenols terphenols, carbohydrates, Phlobotanin, alkaloid. Andantioxidant, antimicrobial activity.Also quantitative Method to indicate presence of antioxidants and total content of flavonoid, ascorbic acid, tannin,total antioxidants are higher in *Carciya papaya*.Compare to *Solanumnigrum,pomengrate,Indian goose berry,Mentha spicata* had lower activity.
- ✓ Antimicrobial activity was identified using different microorganisms'. The microorganisms such as *Klebsilla, Salmonella, Staphylococcus aureus, Streptococcus, Escherichia coli*. The antimicrobial activity is had higher activity from pomengrate and *Carciyapapaya*, compare to other plants extracts.
- ✓ We can use plant disc and different solvent extracts are methanol and hexane to screen the microbial activity of plant extracts. It is higher from activity Mentha spicata and pomengrate compare to *Carciyapapaya,Solanum nigrum*, and *Indian goose berry.*
- ✓ The minimal inhibitory concentration of plant extracts are higher activity from pomengrate. Thin layer chromatography are separate the plant extracts it can be produce the brown colour , black yellow conformation for flavonoid ,caumarrins, quinone.
- ✓ TLC slides are used to bio autography. *Solanum nigrum pomengrate,Mentha spicata ,carciya papaya* had inhibition activity produce red and violet colour we conclude *carciya papaya, pomengrate ,Mentha spicata* had lot of medicinal properties .Further we can say about the plants are used in normal in life only fruits but the leaves are had lot of medicinal properties . This are the plants leaves are used in thousands of years for medicinal uses only.
- ✓ The plant leaves are taken in any type either tea or juice and food consumption. These plants are helps to cardiac disease, iron deficiency, inflammatory lung disease, and autoimmune arthritis, weight reduction, Diabetics.

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