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ANTIBACTERIAL ACTIVITY OF TOTAL FLAVANOID CONTENT OF WHOLE PLANT OF CANAVALIA ENSIFORMIS

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ABSTRACT

Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly are in combination to treat, diagnose and prevent illness or maintain well- being. Countries in Africa, Asia, Latin America use traditional medicine to help meet some of their primary health care needs. In Africa, up to 80% of the population uses traditional medicine for primary health care. In industrialized countries, adaptations of traditional medicine are termed Complementary or Alternative (CAM).

INTRODUCTION

The main classes of antimicrobial agents are disinfectants non selective antimicrobials such as bleach, which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics which are applied to living tissue and help reduce infection during surgery, and antibiotics which destroy microorganisms within the body [1].

The term antibiotic originally described only those formulations derived from living organisms but is now also applied to synthetic antimicrobials, such as sulphonamides, or fluoroquinolones. The term also used to be restricted to antibacterial, but its context has broadened to include all antimicrobials. Antibacterial agents can be further subdivided into bactericidal agents, which kill bacteria, and bacteriostatic agents, which slow down or stall bacterial growth [2].

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Plant Profile:
Canavalia ensiformis

- Kingdom : Plantae
- Order : Fabales
- Family : Fabaceae
- Kingdom : Plantae
- Comman name: Jack bean

Figure 1: Jack bean

Plant Description

Jack bean is a drought-tolerant, fast growing, sometimes shrubby twinning annual to short-lived perennial plant that grows about 50-200cm tall but can become up to 10 meters long when climbing, its stems supporting themselves by twinning around other plants.[3] The plant has the ability to continuously grow under severe environmental conditions, even in nutrient depleted, highly leached, acidic soils. Jack bean is drought resistant and immune to pests. It can grow in poor droughty soils, and does not grow well in excessively wet soil. It will drop its leaves under extremely high temperatures and may tolerate light frosts as well [4]. The plant is woody with a strong, long tap root system. Its roots have nodules which fix nitrogen.

Traditional uses and benefits of jack bean

- In china, the whole plant is pounded and applied to boils.
- Seed is used as stomachic and tonic, also to strengthen the kidney.
- Jack bean seed is used as an antibiotic and antiseptic in Nigeria.
- It is also used for treating ozena, Haemorrhoids, Pyorrhoea, Otitis media, Boils, Cancers, Inflammatory disease and atopic dermatitis in japan.
- Jack bean extract is used in soap for the treatment of athlete's foot and acne in Korea.
- Leaves were used by the Malays in treating Gonorrhoea in Peninsular Malaysia.
- Leaves were used with other substances in a kind of magic tonic that was squeezed into the eyes.
- The plant was pounded and applied to boils.
- The seeds were also used medicinally.
- It is used in the treatment of Vomiting, abdominal dropsy, Kidney related lumbago, asthma, Obesity, stomach-ache, dysentery, coughs, headache, intercostal neuralgia, epilepsy, schizophrenia, inflammatory diseases and swellings in Korea.
- The leaves Plant were dried. It needs to be crushed, using a pestle and mortar, to provide a greater surface area[5].The plant material should be sufficient to fill the porous cellulose thimble (in our experiments we use an average of 14 g of thyme in a 25- x 80-mm thimble).

Extraction

The extraction was carried out by Soxhlet extractor apparatus given them a greater appreciation for the process of extraction, as opposed to testing an antimicrobial compound out of a purchased bottle.We begun by building a rig using stands and clamps to support the extraction apparatus [6]. Following this, the solvent (250 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material is loaded into the thimble, which was placed inside the Soxhlet extractor [7] The side arm was lagged with glass wool. The solvent was heated using the isomantle and was begun to evaporate, moving through the apparatus to the condenser. The condensate then drips into the reservoir containing the thimble. Once the level of solvent reaches the siphon it pours back into the flask and the cycle was begun again. The process should run for a total of 16 hours [8].

Once the Soxhlet extractor apparatus has set up the extraction it can be left to run without direct supervision. It is not advised to leave the equipment completely alone due to the mix of running water and an electrical appliance, so a technician or other lab user should be made aware. The equipment can be turned on and off when overnight running was not permitted, and the time split over a number of days. For good practice, a control should be added.[9]This could be plant material that has known antimicrobial effect at the testing stage.

Chemical Tests

A preliminary phytochemical investigation will be carried out for all the extracts obtained from the medicinal plants. (Brain and Turner, 1975).

Detection of Alkaloids

Small portions of solvent-free chloroform, alcohol and aqueous extracts will be stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate will be tested with various alkaloid reagents.

Mayer's test:

Acidic test solution will be treated with potassium mercuric iodide (Mayer's reagent) and the formation of cream coloured precipitate indicates the presence of alkaloids.

Dragendroff's test:

Acidic test solution will be treated with potassium bismuth iodide (Dragendroff's reagent) and formation of reddish brown precipitate indicates the presence of alkaloids.

Detection of Glycosides

Preparation of test solution

The test solution will be prepared by dissolving extract in the alcohol or hydro-alcoholic solution.

Test for Cardiac Glycosides

Baljet's test:

The test solution will be treated with sodium picrate. Formation of yellow to orange colour indicates presence of cardiac glycosides.

Bromine water test:

Test solution will be dissolved in bromine water. Formation of yellow precipitate indicates presence of cardiac glycosides.

Borntrager's test:

Powdered drug will be boiled with 5 ml of 10% sulphuric acid for 5 mins. Filtered while hot, cooled and the filtrate will be shaken gently with equal volume of benzene. Benzene layer will be separated and then treated with half of its volume solution of ammonia (10%). The ammonical layer with rose pink colour indicates the presence of anthraquinones.

Cyanogenetic glycosides

Grignard's test:

Strips of sodium picrate filter paper will be inserted between split cork stoppers which will be fitted in to the neck of the test tube containing a small amount of powdered drug in water. Care will be exercised that the paper didn't touch the inner side of the test tube. The content will be warmed for half an hour. The red colour of the strips indicates the presence of cyanogenetic glycosides.

Detection of Gums & Mucilage

About 10 ml of aqueous extract will be added to 25 ml of absolute ethanol with constant stirring. Precipitate examined for its swelling properties and for the presence of carbohydrates.

Detection of Flavonoids

Shinoda test:

To the test solutions, a few fragments of magnesium metal will be added along with concentrated hydrochloric acid, and heated. Formation of magenta colour indicates the presence of flavonoids.

In-Vitro Anti-Bacterial Activity

Microbial strains tested

In this study, Microorganisms were selected to cover Gram-positive bacteria and Gram-negative bacteria namely Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Putida bacteremia, Pseudomonas aeruginosa, Bacteroides, Corynebacterium, Sarcina . The tested strains were obtained from Microbiology Laboratory, SVCP, A. Rangampet, Tirupati. The Microorganisms were allowed to grown over night at 37^0 C in 2% nutrient agar at pH 7. The sensitivity of Microorganisms to the reference antibiotic was checked. For this purpose Streptomycin was used as reference antibiotic [13].

Preparation of inocula

The inocula were prepared by inoculating a loop of each bacterial strain from a 24 hours of old culture into a sterile nutrient broth aseptically in the laminar air flow unit. The culture growth was allowed for 24 hours in incubator at 37^0 C [14].

Table 1: Phytochemical analysis of Ethanolic extract of Canavalia ensiformis

S.NO	TEST	EESE
1.	Alkaloids	+
2.	Flavonoids	+
3.	Tanins & phenolic compounds	+
4.	Steroids	+
5.	Terpenoids	–
6.	Cardiac Glycosides	+
7.	Saponins	–
8.	Carbohydrates	+
9.	Amino acids	+
10.	Coumarin glycosides	+
11.	Gums	–

Table2: Antibacterial activity of Ethanolic extract of Canavalia

Microorganisms	Zone of inhibition
	EESE
Putida bacteremia	29±1.0
Bacillus subtilis	31±1.0
Sarcina	27±2.0
Staphylococcus aureus	30±1.0
Escherichia coli	30±2.0
Pseudomonas aureginosa	32±2.0
Bacteroids	22±2.0
Coryenebactrium	21±2.0

Table 3: Evaluation Parameters of Ethanolic Extracts of Canavalia ensiformis

S.No	Physicochemical Properties	Values
1	Determination of Flavonoids value	86%
2	Determination of Total ash Values	25%
3	Determination of Acid Insoluble Ash Value	59.5%
4	Determination of Water Soluble Ash Value	57%
5	Determination of Moisture (Loss on Dryimg)	1.41 g
6	Determination of Crude organic matter	0.984%w/w
7	Determination of Crude Fibre by the Dutch Method	0.32 g
8	Determination of Alcohol Soluble Extractives	0.28g
9	Determination of Water Soluble Extractives	0.24g
10	Determination of Inorganic matters	0.48g

Table 4: Antibacterial activity of standard Streptomycin

Microorganisms	Zone of inhibition
	Standarad (Streptomycin)
Bacillus subtilis	**27±1.0**
Sarcina	**24±1.0**
Staphylococcus aureus	**25±1.0**
Putida bacteremia	**28±2.0**
Pseudomonas aureginosa	**22±2.0**
Escherichia coli	**23±1.0**
Bacteroids	**21±1.0**
Corynebacterium	**22±2.0**

Figure 2: Antibacterial activity of Ethanolic extract of Canavalia

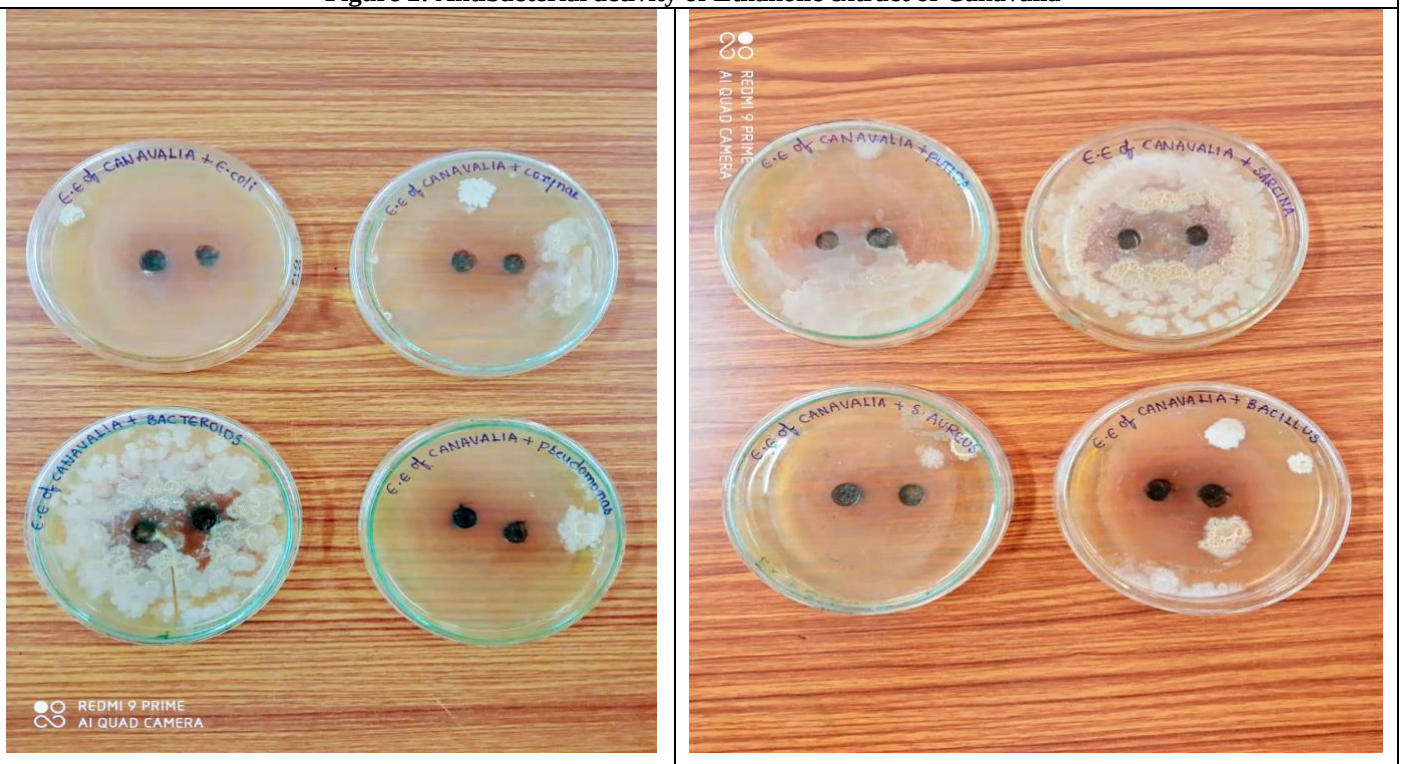
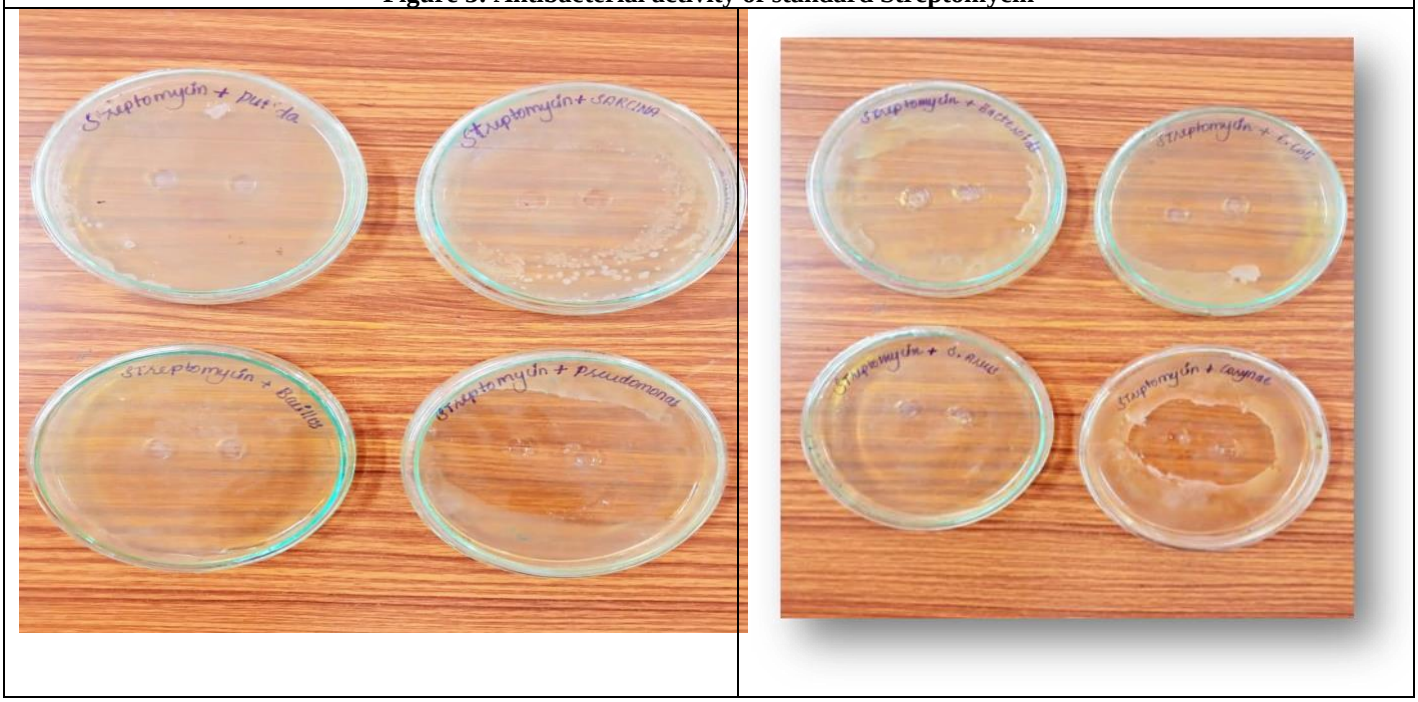


Figure 3: Antibacterial activity of standard Streptomycin



Determination of antibacterial activity

The screening of Anti-bacterial efficacy of the ethanolic extract of Canavalia ensiformis was performed on various microorganisms by using agar well diffusion method (Perez C et al., 1999). The agar plates were prepared by pouring 20 ml of sterile molten Mueller-Hinton (MH) agar (Himedia Lab Pvt. Ltd, Mumbai, India)[15]. The bacterial cultures were prepared by adding the seed culture in the autoclaved agar medium followed by pouring into Petri plates. The solid agar medium was gently punctured with the aid of 8mm sterile cork borer to make a proper well. 50µl of Canavalia ensiformis extract (50mg/ml) was added in the pre labelled wells together with reference antibiotic i.e. Streptomycin [16]. Here ethanolic extract of Canavalia ensiformis served as test and Streptomycin served as standard. The reference Antibiotic was used in the concentration range of 100µg/ml. It was taken care that the sample should be placed at the level of cavity. The diffusion of extract was allowed for 1hr at room temperature on a sterile bench. Then the Petri plates were incubated for 48 hrs at 37^0 C. After 48 hrs the plates were observed for the presence of inhibition of bacterial growth and that was indicated by clear zone of inhibition of bacterial growth around the wells. The size of Inhibitory zone was measured in mille meters (mm). Minimum Inhibitory Concentration (MIC) was determined [17-23].

RESULTS AND DISCUSSION

The results of modified agar well diffusion method (Table 1) showed that prepared EESE, having Inhibitory effect on the microorganisms which are responsible for the intestinal infections Respiratory tract infections and urinary tract infection [24]. The Anti-microbial activity of the herbal extract has been comparable to that of market antibiotic (Streptomycin) .The diameter of Zones of inhibitions was also given in the table 2.

SUMMARY AND CONCLUSION

In the present study an attempt has been made to explore Pharmacognostical and phytochemical parameters besides evaluating antimicrobial activity against microorganisms causing Respiratory tract infection, intestinal infections and urinary tract infection. The identification of plant material taxonomically and Pharmacognostically is important to provide standards and avoid adulteration of drugs. The plants were identified and authenticated by Prof N.Yasodamma, Head of Botany Department, Sree Venkateswara Univerity. Tirupathi.

The detailed botanical, Pharmacognostical studies with proper authentication of the plants helps in minimizing the adulteration and also for proper identification of the plant.

Preliminary phytochemical analysis of the extract showed the presence of the Alkaloids, Flavonoids, Tannins and Phenolic Compounds, Steroids, Cabohydrates, Amino acids, Coumarin glycosides and constituents may be responsible for the healing potential of Respiratory tract infections, Intestinal and Urinary tract infections.

Evaluation of antimicrobial activity of Canavalia ensiformis against microorganisms causing Respiratory

tract infections, intestinal and urinary tract infections was done by using agar well diffusion method. After 24 hrs we measured the zone of inhibition to confirm the antimicrobial activity of the prepared Canavalia ensiformis. From the above results it can be concluded that the Canavalia ensiformis could effectively fight against microorganisms causing Respiratory tract infections, Intestinal and Urinary tract infections.

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Nil

CONFLICT OF INTEREST
No Interest

REFERENCES

1. Weiyan C & Liangzhong Xu. (2014). Research on Antibacterial Activity of Schisandra chinensis extracts by microwave-assisted. Journal of Chemical and Pharmaceutical Research, 6(6), 33-38.
2. Ayandele A & Ayandele OB. (2015). Antibacterial Activity of the Leaf Extracts of Terminalia. International journal of Botany and Research, 5(3), 15-22.
3. Rasna G, Ram Lakhan S, Ankit G. (2019). Antibacterial Activity of the Leaf Extracts of Terminalia bellerica, Terminalia chebula, Emblica officinalis and their formulation Triphala. International journal of Botany and Research, 9(10), 1-8.
4. Antara S and Amla B. (2012). Evaluation of Antimicrobial Activity of different solvent extracts of medicinal plant:Melia azedarach L. International Journal of Current Pharmaceutical Research, 4(2), 67-73.
5. Kannan P, Ramadevi SR. and Waheeta H. (2017). Antibacterial Activity of Terminalia chebula Fruit Extract. African Journal of Microbiology Research, 3(4), 180-184.
6. Usman AK, Hazir R, Zeeshan N, Muhammad Q. (2013). Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. European journal of amicrobiology and immunology, 3(4), 272-274.
7. Tahir J, Muhammad A, Akash T, Basreen A. (2015). Antimicrobial activity of Three medicinal plants (Artemisia indica, Medicago falcata and Tecoma stans). Afr J Tradit Complement Altern Med. 12(3), 91-96.
8. Pugalenthi M, Doss A, Rajendrakumaran D and Vadivel V. (2010). Antibacterial activity of Canavalia ensiformis L. DC Seeds due to solvent extract and fraction method. Indian Journal of Science and Technology, 3(1), 94-95.
9. Vadivel V. (2019). The Nutritional and Antioxident contents of wild Jack bean (Canavalia ensiformis L. DC.) An Under-Exploited Legume from south India. International Journal of Recent Scientific Research, 10(10), 35502-35508.
10. Anja K, Sasa P, Barbara J, Sonja SM. (2010). Evaluation of diffusion and dilution methods to determine the Antibacterial activity of plant extracts. Journal of Microbiological methods, 22, 121-126.
11. Glaucia VFM, Aletheia LS, Claudia P. (2012). Antiproliferative effects of lectins from Canavalia ensiformis and Canavalia brasiliensis in human leukemia cell lines. Toxicology, 26, 1161-1169.
12. Rachel NU. (2003). Effect of aqueous extract of Canavalia ensiformis seeds on hyperlipidaemia and hyperketonaemia in alloxan-induced diabetic rats. An International journal published by the Nigerian Society for Experimental Biology, 15(1), 7-15.
13. Eke CNU, Asoegwu SN and Nwandikom GI. (2007). Some physical properties of Jackbean seed. Agricultural Engineering International, 9, 1-11.
14. Somayeh R, Mahmood A, Salmah B. (2014). Antibacterial activity of leaf extracts of Baeckea frutescens against Methicillin-Resistant Staphylococcus aureus. Bio Med Reasearch International, 2, 1-5.
15. Nayan RB & Shukla VJ. (2015). Antibactrial and Antifungal activities from leaf extracts of Cassia fistula L. An Ethnomedicinal Plant, Journal of advanced Pharmaceutical Technology and Research, 2(2), 104-109.
16. Gislene GF, Nascimento JF, Paulo CF, Gauliana LS. (2000). Antibacterial activity of plant extracts and Phytochemicals on Antibiotic-Resistant Bacteria. Bio Med Reasearch International, 31, 247-256.
17. Jony M, Hafsha BC, Abdullah A. (2013). Pharmacological profile of Catharanthus roseous, Asian journal of pharmaceutical research and development, 3, 45-50.
18. Prakash N, A.Elumalai, C.Soundarajan. (2017). Study of Antimicrobial activity of Chloris barbata leaves s. International journal of pharmaceutical and applied Sciences, 2(1), 37-40.
19. Sakala B, Medarametla N, Mahesh B, Gopisetty S and Sreekanth N. (2013). An evaluation of the Antibacterial activity of roots extracts of Chloris barbata against Staphylococcus aureus and Escherichia coli, International journal of biopharma research, 31, 100-101.
20. Damles and Sharon K. (2017). Phytochemical studies of Ixora coccinea Linn. International journal of Biology, Pharmacy and Allied Sciences, 6(7), 1403-1415.
21. Mani M, Marimuthu C, Uma M, et al. (2014). Antimicrobial activity and phytochemical screening of various parts of Ixora coccinea plant. Journal of medicinal plant research, 8(10), 423-429.

22. Saleha A, Tahmina H, Esrath J, Faisal K, et al. (2015). Comparative antimicrobial activities of different species of Ixora. Journal of Pharmacognosy and Phytochemistry, 3(6), 103-105.
23. Annapurna PV, et al. (2002). Antimicrobial activity of Ixora coccinea plant, Elsevier.
24. Nithiyasoundari M, Parimala KS, Seeli B. (2015). Efficacy of Ixora coccinea against common fish pathogens. International journal of research in Ayurveda pharma, 6, 4.