IJCRCPS

International Journal of Current Research in Chemistry and Pharmaceutical Sciences
www.ijcrcps.com *Volume 1 Issue 1. 2014 Pages: 45-55*
(pISSN: 2348 – 5213; eISSN: 2348 - 5221)

RESEARCH ARTICLE

IDENTIFICATION OF VIBRIOCIDAL COMPOUNDS FROM HERBAL PLANT USING TLC AND BIOAUTOGRAPHY

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Abstract

The present investigation on the antimicrobial activity of medicinal plants leaves *viz., Solanum nigrum*, *Psidium guajava*, *Aegle marmelos, Acorus calamus*, and *Syzgium cumini.* Against microbes is summarized here. Bacterial cultures of *Vibrio cholerae, V. vulnificus, V. parahaemolyticus, V. alginolyticus* were obtained from Department of microbioloty. Tamil nadu, India for testing them against the above medicinal plant leaves. Disc diffusion method was employed to find out the antibacterial activity of the leaves against the microbes used, different solvents *viz.,* Ethyl acetate, water and methanol were used in preparation of different plant extract. Among the solvent used water extract of the leaf possessed more antibacterial activity (vibriocidal activity) than the water, ethyl acetate and methanol extract.
Key words: medicinal plants, *Vibrio cholera,* Disc diffusion method.

Introduction

The roots of Indian medicine were set forth in the sacred writing called the Vedas, Which date back as far as the 2nd century BC The Indian system of medicine was called the Ayurveda. The Indian material medic or list of herbs used as Medicines was quite extensive. As early as 800 BC one Indian Writer know 500 medicinal plants and another knew 760 –all indigenous plants of India Indian Herbalism of Ayurveds is still practiced today and many authentic, traditional formulations are Available is still practiced today and many authentic, traditional formulations are Available outside of India **(Iwu *et al.,* 1999).**

Medicinal plant from a large group of important flora plants provide basic raw materials for the indigenous pharmaceutical Industries such as medicinal, cosmetic perfumery and food etc. The medicinal plants are referred to plants that are used for their therapeutic or medicinal values. The whole plant or its different parts may be valued for its therapeutic medicinal aromatic or savory qualities. These plants produce and contain a variety of chemical substances that act upon the human body. It also helps country to earn some valuable foreign exchange. Industrial sources reveal that Ayurveda and Unani the two systems of herbal medicines alone have pegged an amount of foreign exchange of 220 crores in 2000-2001 which is nearly 20 percent of the business. Developed countries like Europe, Russia, USA, Australia and Japan covering the spectrum of as many as 127 countries are most potential destinations for our products **(Elnima *et al.,* 1983).**

The use of the leaves, flowers, Stem, berries and roots of the plants are known to

IJCRCPS

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www.ijcrcps.com *Volume 1 Issue 1. 2014 Pages: 45-55*
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prevent, relieve and treat illness. They also play vital role as an Antmicrobial agent **f**rom a scientific perspective, many herbal treatments are considered experimental. The reality is however, that herbal medicine has a long and respected history, there has been resurgence in the consumption and demand for medicinal plants Today science has isolated the medicinal properties of a large number of botanical and their heating components have been extracted and analyzed. Many plant components are now synthesized in large laboratories for the use in pharmaceuticals preparations. Resistant to Antimicrobial agents such as antibiotics, is emerging worldwide for variety of organisms and multiple drug resistant organisms pose serious threats to treat infectious diseases. Hence plant derived Antimicrobials have received considerable attention in recent years**.**

India is endowed with about 47000 species of plants and ranks 8th in the world biodiversity, out of these, 8000 species are known to be medicinal. Indian system of medicine use around 2,500 plant species belonging to more than 1000 genera. About 8000 species are used by industries of which approximately 25% are presently cultivated. Similarly, some 1300 plant species are known to contain aroma but only about 2% are grown. India, being country wide geographical variations, has vast potential to grow these plants Moreover cultivation of these plants can offer a wide scope for small farmers to improve their living standards. There is an urgent need for development of medicinal and aromatic plants through organized cultivation to meet the growing national and international demand in substantial quantities, to support user industry and dispensaries based on Indian system of medicine **(Jones *et al.,* 1997)**

The major Virulence factor produced by *V.cholerae* is the cholera enterotoxin (CT).encoded by tow contiguous genes contiguous genes forming the ctx AB operon. Although most environmentally isolated *V.cholerae* 01 strains do not carry the ctx AB operon These strains, which are distinct from the indigenous strain, have recently been isolated frome shellfish and finfish (**Kapet *et al* .,1982)**.**Sakazaki 1983** observed that the *Vibrio parahaermolyticus* can grow in the process of 1 to 8% NaCl best growth in the 2 to 4% range. It dies of f in distilled water **(Bean *et al.,* 1990).** It does not grow at 4ºC, but growth between 5^{0}c and 9^{0}c has been demonstrated at pH 7.2 to 7.3 and 3% NaCl or at pH 7.6 and 7% NaCl. Its growth 9ºC to 10ºC in food products has been found to be 10ºC **(Beuchet, 1987).** The upper growth temperature is 44ºC with an optimum between 30ºC and 35ºC.

The Antmicrobial properties of plants have been investigated by number of researchers world wide, especially in Latin America. In Argentina, a researcher tested 122 known plant species used for therapeutic treatments.

Materials and methods

Plant samples used

Psidium guajava a small trees and large shrubs. Leaves are ovate. Elliptic, paberulous and leathery. Flowers are white and fragrant. Fruits are green to light yellow to a pulple.

Preparation of leaf extracts

The preparations of different leaf extracts were done through modified method (Priya and Ganjewala, 2007).

Processing of samples

Isolation of Bacteria from samples

Using sterile swabs samples were collected from the shrimp, in particular from areas such as fish, all the fish specimens were rinsed with stele distilled water to remove the allaying on the Samples.

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Evaluation of antimicrobial activity of medicinal plants

Antimicrobial activity of medicinal compound is tested through several methods like tube dilution methods, well plate methods and disc diffusion methods. Disc diffusion method is most commonly employed methods to evaluate the antimicrobial activity. In the antimicrobial activity of leaves of *solanum nigram, psidium guajava, Acorus colamus, Syzgium cumini and Aegle malmelos.*

Preparation of inoculums

The organism to be used for the in vitro were maintained and preserved on nutrient agar slopes by refrigeration at 4^{o} C Subcultures were dine at regular intervals. Nutrient broth was prepared to about 5 ml on nutrient broth a lapful of Culture was inoculated and incubated at 37^{o}C for 24 hours. The microbial suspension was now used for antimicrobial activity.

Preparation of paper disc

Disc of 5 mm diameter were prepared using what man filter Paper no.1. These sterilized in the hot air oven at 50^{o} Cfor 1 hour. The discs were impregnated with 20 ul of different solvent extracts (whale methanol ethyl acetate) at 100 mg ml concentration for the five different leaves to check their antimicrobial activity .the sterilized paper disc with different solvent (separately) were used as control.

Antimicrobial susceptibility test

Disc diffusion method was adopted for evaluation of Antimicrobial activity of five different medicinal leaves. Muller Hinton agar was prepared and autoclaved at 151 b pressure for 20 minutes and cooled at 45^{o}C. The cooled media was poured on to sterile Petri plates and allowed for solidification. The plates with media were leaf extracts with the respective microbial suspension using sterile swab. The disc impregnated with respective leaf extract at 100 ml concentration individually were placed. On the four corners of each Petri dishes control disc was also placed. The parasites were then incubated at 37^{o}C for 24 hours after incubation period the diameter of the zone formed around the paper disc were measured and expressed in mm.

Identification of vibriocidal compounds from herbal plants using TLC and BioAutography

The components, visible as separated spots, are identified by comparing the distances they have traveled with those of the known reference materials. Measure the distance of the start line to the solvent front (=d). Then measure the distance of center of the spot to the start line (=a). Divide the distance the solvent moved by the distance the individual spot moved. The resulting ratio is called R_f-value. The value should be between 0.0 (spot did not moved from starting line) and 1.0 (spot moved with solvent front) and is unitless.

TLC – BioAutography

The inoculum of *Vibrio* spp. Containing 106 CFU ml-1 in molten muller Hinton agar was distributed over. The already prepared TLC plate. After solidification the suspension the TLC – bioautography plate was incubated at 37C for 24 hrs. The bioautogram developed was sprayed with 1% aqueous solution (MDT) and incubated at 37C for 24 hrs. Inhibition zones indicated the presence of active compound. Growth inhibition areas were compared with the Rf of the related to spots on the reference TLC plate.Preparative TLC plates with the thickness of 1mm were prepared using same stationary and mobile phase as above with the objective of isolating the compounds of plant extract that inhibited the growth of *Vibrio* spp.

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Results

The results of the experiment describe the antimicrobial effect of plant extract of leaves of *Solanum nigrum. Psidium gujava, Syzgium cumini, Acorus calamus, and Aegle marmales* with different solvents viz., water, methanol and Ethyl acetate against *vibrio* sps isolated from fish and prawn samples. The antimicrobial activity was studied by disc diffusion method and the antimicrobial activity was measured by zone of inhibition in millimetes formed alound the disc. The **TLC**-bioautography analysis was also studied for the identification of compounds (Table 1)

The five leaf extracts of *Psidium guajava* at 100mg/ ml recorded inhibitory activity against *V. cholera, V. parahaemolyticus, V. vulnificus,* and *V. alginolyticus,* which was compared with the control. Methanol extract recored more inhibitory effect than water and Ethyl acetate. The water extracts of *Psidium guajava* at 100mg/ml recorded the diameter of inhibition zone (10mm) for *V. cholera*, and *V. parahaemolyticus* followed by (16mm) for *V. vulnificus* (12mm) *and V. alginolyticus* (13mm).

The methanol extract of *Psidium guajava* at 100mg/ml recored the diameter of inhibition zone (17mm) for *V.vulniificus* followed by (16mm) for *V. parahaemolyticus* followed by (14mm) for *V. cholerae* followed by (14mm) for *V. alginolyticus.* Ethyl acetate extract of *Psidium guajava* 100mg/ml recorded the diameter of inhibition zone (9mm) for *V. cholera* followed by (9mm) for *V. parahaemolyticus* followed by (7mm) for *V. vulnificus* followed by (6mm) for *V. alginolyticus,* in diameter.

The five leaf extract of *solanum nigrum* at 100mg/ml recorded inhibitory activity against *V. cholerae, V.parahaemolyticus,v.vulnificus,*and *V alginolyticus* which was compared with the control. Methanol extract recorded more inhibitory effect than water and ethyl acetate extract. The water extracts of *solanum nigrum* at 100mg/ml recorded the diameter of inhibition zone (13mm) for *V. vulnificus, V .cholerae, V. parahaemolyticus* followed by (8mm) for *V. alginolyticus* was no activity.

The water extract of *Acorus calamus* at 100mg/ml recorded the diameter zone (13mm) for *V. parahaemolyticus* followed by (12mm) for *V. alginolyticus* and *V. cholerae* followed by (9mm) for *V. vulnificus* (8mm). The methanol extract of *Acorus calamus* at 100mg/ml recorded the diameter zone of (25mm) for V. *parahaemolyticus,* followed by (20mm) for *V. cholerae*, and *V. alginolyticus,* followed by (23mm) for *V. vulnificus*. The ethyl acetate extract of *Acorus calamus* at 100mg/ml recorded the diameter zone of [10mm] for *V. cholerae*, followed by (5mm) for *V. parahaemolyticus,* and there is no zone formation against other organisms.

The five leaf extract of *Aegle marmolos* at 100mg/ml recorded inhibitory activity against *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus* which was compared with the control. Methanol extract recorded more inhibitory effect that water and Ethyl acetate extract. The water extract of *Aegle marmelos* at 100mg/ml recorded the diameter zone of (12mm) for *V. alginolyticus,* followed by (8mm) for *V. vulnificus* and *V.cholerae* (8mm) for *V. parahaemolyticus* is no zone formation.

The methanol extract of *Aegle mormelos* at 100mg/ml recorded the diameter zono of (15mm) for *V. cholerae,* followed by (15mm) for *V. parahaemolyticus* and *V. vulnificus* followed by (15mm) for *V. alginolyticus* (14mm). The ethyl acetate extract of *Aegle marmelos* at 100mg/ml recorded the diameter zone of (8mm) for *V. cholerae*, followed by (6mm) for *V. vulnificus*

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TABLE. 1 Antibacterial activity of crude leaf extract i in comparison to different extraction solvent at concentration [100 mg / 1ml]

S.No	Solvent used for extraction	Micro organisms	Zone of inhibition In millimeter	
			Standard	Concentration in 100mg/ml
1.	Water extract	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	25mm 15mm 20mm 20mm	10mm 16mm 12mm 13mm
2.	Methanol extract	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	25mm 15mm 20mm 20mm	14mm 16mm 17mm 14mm
3.	Ethyl acetate	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	25mm 15mm 20mm 20mm	9mm 9mm 7mm 6mm

Figure. 1 Antibacterial activity of methanol extract of *Psidium guajava* to different extraction solvent at concentration 100mg/ml

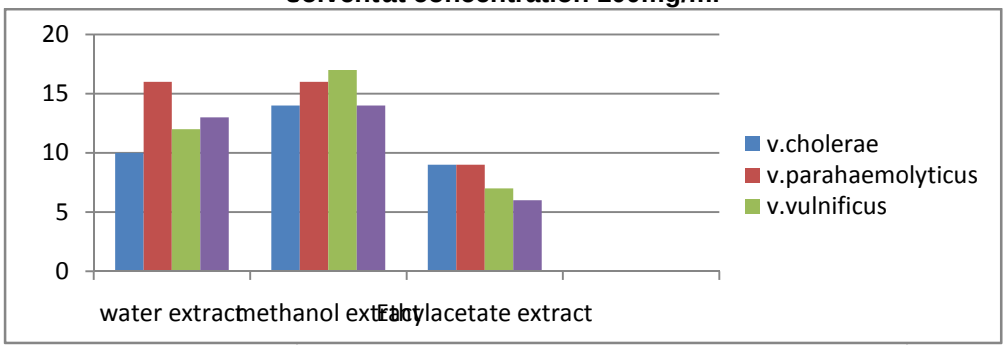

Table. 2 Antibacterial activity of methanol leaf extract of *Solanum nigrum* in comparison to different extraction solvent at concentration 100mg/ml.

S.No	Solvent used for extract	Micro organisms	Zone of inhibition in millimeter	
			Standard	Concentration in 100mg/ml
1.	Water extract	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	22mm 15mm 20mm 19mm	8mm 8mm 13mm
2.	Methanol extract	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	22mm 15mm 20mm 19mm	9mm 13mm 15mm 17mm
3.	Ethyl acetate	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	22mm 15mm 20mm 19mm	10mm 8mm 8mm 7mm

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Figure.2 Antibacterial activity of crude leaf extract of *solanum nigrum* to different extraction solvent at concentration (100mgml)

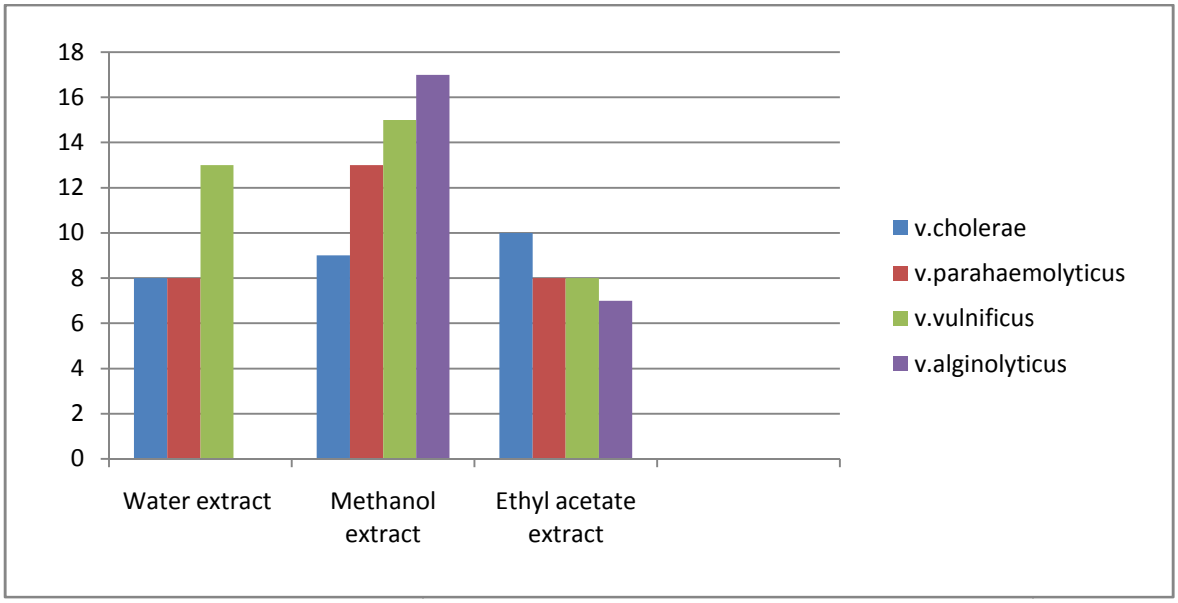

Table. 3 Antibacterial activity of crude leaf extract of *Syzgium cumini* in comparison to different extraction solvent at concentration [100mg/ml].

S.No	Solvent used for extraction	Micro organisms	Zone of inhibition in millimeter	
			Standard	Concentration in 100mg/ml
1.	Water extract	*v.cholerae*	15mm	mm
		v.parahaemolyticus	20mm	13mm
		v. vulnificus	18mm	8mm
		v. alginolyficus	20mm	12mm
2.	Methanol extract	*v.cholerae*	15mm	5mm
		v.parahaemolyticus	20mm	10mm
		v. vulnificus	18mm	5mm
		v. alginolyficus	20mm	6mm
3.	Ethyl acetate extract	*v.cholerae*	15mm	10mm
		v.parahaemolyticus	20mm	7mm
		v. vulnificus	18mm	7mm
		v. alginolyficus	20mm	10mm

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Figure. 3 Antibacterial activity of crude leaf extract *Syzgium cumini* to different extraction solvent at concentration [100mg/ml]

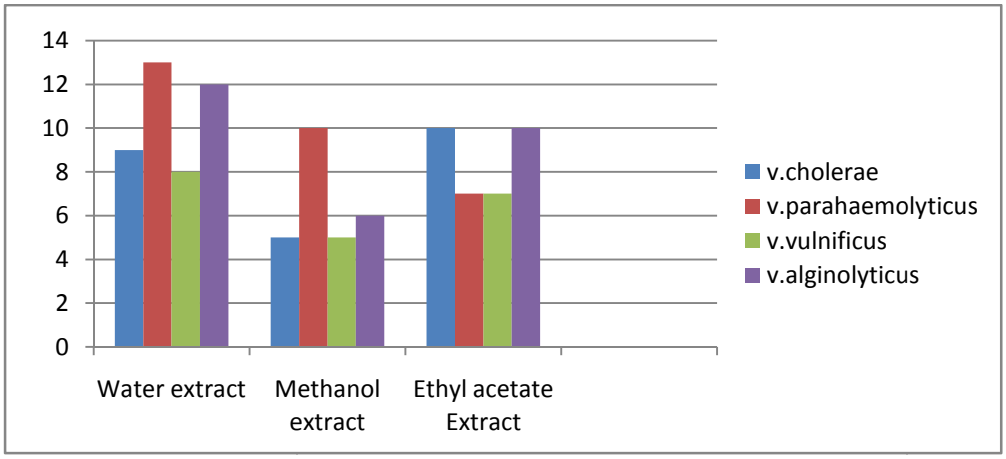
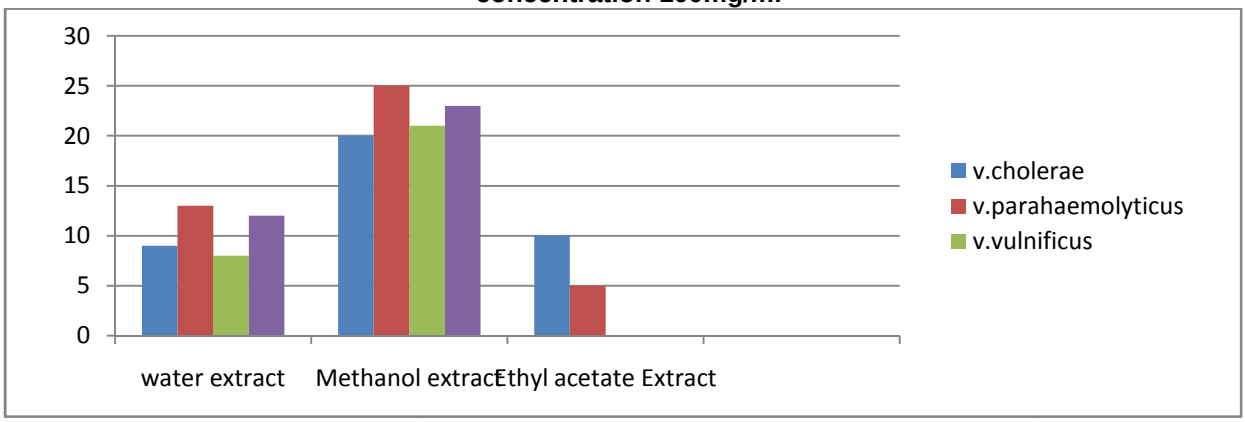


Table. 4 Antibacterial activity of crude extract of *Acorus calamus* in comparison to different extraction solvent at concentration [100mg/ml]

S.No	Solvent used for extraction	Micro organisms	Zone of inhibition in millimeter	
			Standard	Concentration in 100mg/ml
1.	Water extract	*v.cholerae*	25mm	9mm
		v.parahaemolyticus	15mm	13mm
		v. vulnificus	20mm	8mm
		v. alginolyticus	20mm	12mm
2.	Methanol extract	*v.cholerae*	25mm	20mm
		v.parahaemolyticus	15mm	25mm
		v. vulnificus	20mm	21mm
		v. alginolyticus	20mm	23mm
3.	Ethyl acetate	*v.cholerae*	25mm	10mm
		v.parahaemolyticus	15mm	5mm

Figure. 4 Antibacterial activity of crude extract of *Acorus calamus* to different extraction solvent at concentration 100mg/ml



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www.ijcrcps.com Volume 1 Issue 1. 2014 Pages: 45-55
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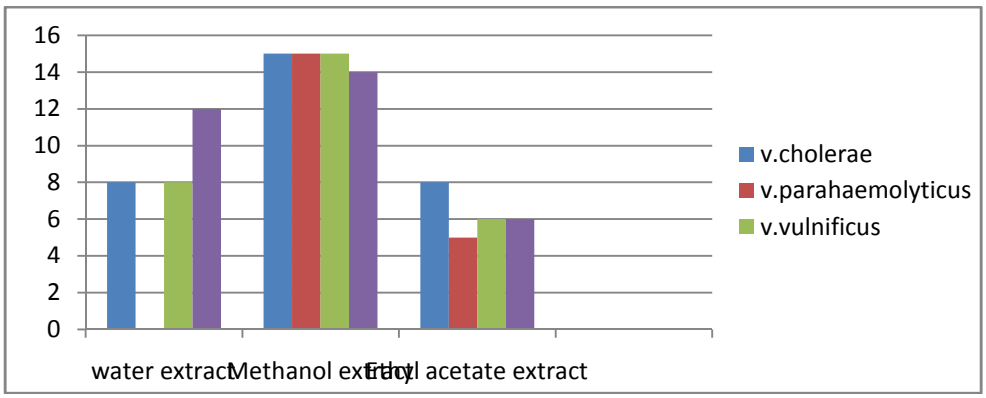
Table. 5 Antibacterial activity of crude leaf extract *Aegle marmelos* in comparison to different extraction solvent at concentration [100mg/ml].

S.No	Solvent used for extraction	Micro organisms	Zone of inhibition in millimeter	
			Standard	Concentration in 100mg/ml
1.	Water extract	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyficus*	22mm 15mm 20mm 19mm	8mm 8mm 12mm
2.	Methanol extract	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyticus*	22mm 15mm 20mm 19mm	15mm 15mm 15mm 14mm
3.	Ethyl acetate	*v.cholerae* *v.parahaemolyticus* *v. vulnificus* *v. alginolyficus*	22mm 15mm 20mm 19mm	8mm 5mm 6mm 6mm

Figure . 5 Antibacterial activity of crude leaf extract of *Aegle marmelos* to different extraction solvent at concentration [100mg/ml]



and *V. alginolyticus* followed by (6mm) for *V. parahaemolyticus*.

Discussion

Recently, much attention has been directed towards plant ertracts and biologically active compounds isolated from popular plant species. The use of medicinal plants play a vital role in covering the basic health needs in developing countries and these plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms.

Vibriocidal properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plants extract of their active constituents are used as Folk medicine in traditional therapies of 80% of the world population. There are about 45,000 plants species in India with capacity to produce a large number of

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www.ijcrcps.com *Volume 1 Issue 1. 2014 Pages: 45-55*
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organic chemicals concentration hotspot in the region of eastern Himalayas, of high structural diversity **(Aravindhan *et al.*, 2009).**

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The First step towards this goal is the in virto antimicrobial activity assay and in recent years several reports available on antimicrobial activity of plant extract a human pathogenic microorganisms.

Plants are known to contain innumerable biologically active compounds. Essential oils occur in five families and many of them have been reported to posscers biological activity such as antifungal antibacterial and insect repellent. There are easily extracted for the plant tissue without any changes in active compounds in composition.

On the basis of the result obtained in this present investigation methanolentract *Psidium guajaver* showed higher to the tested bacteria such as *V.cholera, V. parahaemolytiws, V. vulnificus* and *. alginolyticus.* The antibacterial activity *Solanum nigrum, Syzgium cumini, Aegle marmelos,* and *Acorus calamus* showed more or less equal zone of inhibition or slightly greater against some pathogens when compared to each other.

The solvent methanol, Ethyl acetate and water extract from the leaves to showed more or less equal zone of inhibition or slightly greater against the pathogens. But all results showed *psidium guajava* to have higher activity followed by *solanum nigrum, Acrous calamus, Aegle mermelvs, Syzgium cumini* towards the microbes used. This study also shous the present of different TLC with biological activity that can be of valuable therapeutic index. The results of bioautography in the present investigations showed that all the leaves more or less contain same components.

The chemical constituents of the plant extracts were analysed by thin layer chromatography (TLC), the vibriocidal compounds were determined by TLC. Bioautography and were further confirmed by high performance liquid chromatograply [HPLC]. Significant inhibitory activity was observed with methanol antract of plants against the test bacteria while less antibacterial activity was observed in water, methanol, Ethyl acetates.

The TLC –bioautography analysis showed that gallic acid and tannin present in water extract of *S. cuwmini* tannin present in *Solanum nigrum* and gallic acid present in *Acorus calamus*, *Aglemarmalos, Syzgium cumini*, can be used for the treatment of gasteroenteritis, Diarrhoea and cholera disease. After detailed investigations. We also conclude that the plants in gallic acid and tannin can be used as an alternative to search for new vibriocidal drugs **(**Anjana sharmer *et al.*, 2008).

The present observation suggests that the organic solvents extraction was suitable to verity the antimicrobial properties of medicinal plants and they supported by many investigation. The present study justifies the claimed uses of leaves in the traditional system of medicine to treat various infections diseases caused by the microbes. This study also encourages cultivation of the highly valuable plants in larger scale to increases the economic status of the cultivators in the country. The obtained results may provide or support to use of the plant in traditional medicine. TLC-Bioautography investigations can be done to isolate and identity minor in the leaves and to screen other potential bioactivities may be recommended.

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