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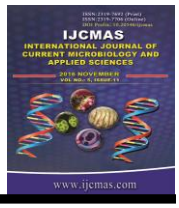

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Extraction of Phytochemicals from Local Selected Plants and their Antibacterial Role

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A B S T R A C T

The present study is carried out to assess the quality of five plants, *Coriandrum sativum, Mentha piperita, Tridax procumbens, Ocimum tenuiflorum* and *Catheranthus roseus* for their phytochemical composition as medicine & their anti-bacterial effects. Phytochemicals of plants were obtained in aqueous & ethanolic extracts. These extracts are screened for the detection of secondary metabolites. Among five selected plants, aqueous and ethanolic extracts of leaf & stem have shown the presence of carbohydrates, alkaloids, steroids, glycosides, flavonoids, terpenoids and also oils & fats. But carbohydrates in leaves of *Mentha* & glycosides in leaves of *Tridax* of ethanolic extracts were found absent. Quinones were absent in both aqueous & ethanolic extracts except in the aqueous extract of stem of *Ocimum*. Tannins were found in both the extracts, but showed negative result in ethanolic extract of leaf of *Ocimum*. Quinones were absent in ethanolic extraction leaf & stems of all plants, but present only in the aqueous extract of stem of *Ocimum*. Tannins found in both aqueous & ehanolic extractions of stem & leaves of all plants except in the ethanolic leaf extract of *Ocimum*. Saponins found present in all but absent only in the ethanolic extract of stem of *Tridax*. Phenols found absent in aqueous extract of stems & ethanolic extraction of leaves of all plant, whereas they are found in aqueous extraction of all leaves except *Tridax*. Antimicrobial activity has shown the highest zone of inhibition by leaf extract of *Vinca roseus* (1.0 cm) on *E.coli* bacteria.

Introduction

Since ancient times, people have been exploring large number of medicinal plants with disease curative properties. Peoples have applied herbs and its derivatives as therapeutic medicines (Newman, *et al*., 2003). Despite the abundance and advancement of synthetic drugs, a significant proportion of population of developing countries still depend on traditional medicines for their health care needs (Lesney, 2004; Okigbo & Mmeka, 2006). Nearly 80% of the world's population relies on traditional medicines most of which involve the use of plant extracts (Schinor *et al*., 2007; Patel *et al*., 2011) and a large number of African and Asian populations use traditional medicines for their healthcare.

In India, about 95% of the prescriptions are plant based in the traditional systems of Unani, Ayurveda, Homeopathy and Siddha (Satyavati & Tandon, 1987). Around 80% of products are of plant origin (Patwardhan & Chorghade, 2004). Hence plants are the richest source of traditional & modern medicines, food supplements, pharmaceutical intermediates etc. and relatively have lower incidences of adverse reactions, reduced cost is encouraging for both the consuming public & consider plants medicines as alternatives to synthetic drugs (Nair *et al*., 2005; Sitara & Hassan, 2011). The chemical substances produced by plants which have a definite physiological action on human body are generally known as phytochemicals. These are non-nutritive and act like shield against diseases associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing (Zheng & Quin, 2007). Nowadays in the developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but are also often with adulteration and side effects (Essawi & Srour, 2000; Shariff, 2001) but medicinal plants are very ancient and only true natural medicines useful in several ways for the treatment of different diseases.

Only a small percentage of all plants species have been studied to some extent for the presence of primary & secondary metabolites (Verpoorte *et al*., 2000) known as phytochemicals. These phytochemicals include both primary metabolites (carbohydrates, proteins, nucleotides etc.) essential for growth, development & survival of the plant, and secondary metabolites (alkaloids, flavonoids, tannins, glycosides, steroids, saponins, resins, phenolic compound, lignins, stilbenes, betalains) required for specific functions like resistance against pests, diseases & herbivores or attraction of pollinator etc (Zheng 2001; Cai *et al*., 2003). These secondary metabolites are not required by the human body for life sustenance, but they offer protection against pathogens (Beegum & Devi, 2003; Kokate *et al*., 2006; Okigbo & Igwe, 2007). Medicinal plants are increasingly gaining acceptance even among the literates, probably due to the increasing in efficacy of modern drugs used for the control of many infections as well as increase in resistance by several bacteria to various antibiotics & the increasing cost of prescription drugs, for the maintenance of personal health. Hence the phytochemical study is an effective approach for discovering new drugs from medicinal plants (Duraipandiyan *et al*., 2006; Razia *et al*., 2013).

Materials and Methods

Collection and processing of plant sps.

Locally available five herb plant species were randomly selected for the extraction of secondary metabolites.

Plants selected for the study

Five local plants were selected such as Coriander (*Coriandrum sativum),* Pudeena (*Mentha piperita),* Tridax daisy (*Tridax procumbens),* Tulsi (*Ocimum tenuiflorum)* and Vinca roseus (*Catharanthus roseus).* They were brought fresh, early in the morning between 6-7am in and around Bidar city during the month of March & April, 2015.

Extraction of phytochemicals for qualitative tests

The Plant parts such as leaves (L) & stem (S) were washed with tap water to remove surface impurities, & then rinsed with

distilled water. All plant parts were made into chopped to 1-2 cm, dried at 60^{o}C & made fine powder using grinder. Two methods (Blessy *et al*., 2012; Sanjay *et al*., 2013) followed for the extraction of phytochemicals qualitative analysis

Aqueous extract: 20gm of each powder sample was soaked & squeezed in 200ml of distilled water for 30 minutes at 70^{o}C. These were filtered through Whatmann filter paper No.1 to get filtrates. These were stored in sterile bottles at 4^{o}C for further use.

Ethanolic extract: 20gm of each powder sample was macerated in 95% ethanol for 72 hours at 27 ^{o}C. These were filtered through Whatmann filter paper No.1 to get filtrates. These were stored in sterile bottles at 4^{o}C for further use.

Detection of phytochemicals by qualitative analysis

Each of leaf & stem extracts was screened for the presence of various phytochemicals by the following qualitative analysis.

Molisch's test for carbohydrates: In 3ml of extract, 2ml of Molisch's reagent was added, shaken, and then 2ml of concentrated H_2SO_4 was poured in it along the side of test tube. The violet ring at the interphase indicated the presence of carbohydrates

Mayer's test for alkaloids: In 2ml of extract, 2ml of concentrated HCl & then 6 drops of Mayer's reagent were added. The pale yellow precipitate indicated the presence of alkaloids.

Ring test for steroids: In 0.5ml of the extract, 3ml of chloroform was added & filtered. The filtrate was mixed with equal amount of concentrated H_2SO_4. The reddish brown colour ring with a slight greenish fluorescence indicated the presence of steroids.

Salkowski's test for glycosides: In 2ml of each extract, 2ml of chloroform, & then 2ml of concentrated H_2SO_4 were added & shaken gently. The reddish brown colour indicated the presence of steroids.

Lead acetate test for flavonoids: In 1ml of extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate indicated the presence of flavonoids.

Ring test for terpenoids: In 2ml of extract, 2ml of acetic acid & 1ml concentrated H_2SO_4 was added. The blue green ring formation indicated the presence of terpenoid.

Concentrated HCl test for quinones: In 1ml of extract, 5ml of concentrated HCl was added. The yellow coloured precipitate indicated the presence of quinines.

Lead acetate test for tannins: In 2ml of extract, few drops of 1% Lead acetate was added. The yellowish precipitate indicated the presence of tannins.

Frothing test for saponins: In 2ml of extract, 2ml of distilled water was added & shaken well. Frothing indicated the presence of saponins.

Ninhydrin test for proteins: In 2ml of extract, 4-5 drops of Ninhydrin reagent was added, mixed & boiled in water bath for 2-3minutes. A bluish-black colour indicated the presence of proteins.

Millon's test for amino acid: In 2ml extract, 4-5drops of Millon's reagent added & heated gently. A reddish-brown colouration/ precipitate indicated the presence of tyrosine residue.

Ferric chloride test for phenol: In 1ml of

extract, 1ml 3% ferric chloride added. The deep blue colour indicated the presence of phenol.

Fehling's test for reducing sugars: In 2ml of extract, 1ml of Fehling's reagent added & the mixture was boiled in waterbath. The brickred colour indicated the presence of glycosides.

Boiling test for Phlobotannins: In 2ml of extract, 2ml of 1% HCl was added & the mixture boiled in waterbath. A red precipitate at the bottom indicated the presence of Phlobotannins.

Spot test for fixed oils and lipids: 2-3 drops of each extracts are passed between two filter papers & allowed to dry. Appearance of an oil stain or a grease spot on the filter paper observed under sunlight indicated the presence of fixed oils.

Extraction of phytochemicals from plants for TLC

Ethanolic extraction method is a modified method of (Abdulrahman *et al*., 2004) used for the extraction of phytochemicals from plants. The fresh parts of the plant were dried in oven & ground to fine powder by grinder. 10 gm of each plant part was then macerated in 100 ml of ethanol for 72 hours, then covered with aluminium foil & labeled. After 72 hours of extraction, each extract was filtered through Whatman's filter paper no.1. The filtrate was evaporated to dryness at room temperature & stored at 5°C in refrigerator (Sanjay *et al*., 2013).

Preparation of TLC plates & loading of samples

The glass plates (20x20cm) coated (0.2-0.3mm thick) with silica gel (30gm/60ml distilled water) were dried at room temperature. The dried plates were activated at 100°C for 30 minutes in an oven & cooled at room temperature. Each sample were separately spotted 1cm above the edge of the plates by using capillary tubes. These spotted glass plates were placed in an air tight chromatography chamber containing 200ml of different solvent mixture/mobile phase for isolation of chemical compounds (Vidya *et al.*, 2008). The developed chromatograms were air dried & one of the TLC plate observed under UV-transilluminator for detection of flavonoids. Remaining each plate sprayed with different reagents (table 1) & air dried for the detection of other metabolites.

Calculation Rf for detection of phytochemicals

The R*f* (=Retention factor) values were calculated for separated bands. Rf is the ratio of distance travelled by sample & distance travelled by the solvent system.

$$Rf = \frac{\text{Distance travel by solute}}{\text{Distance travel by solvent}}$$

Methanolic extraction to assess antimicrobial activity

The leaves & stems washed thoroughly to remove surface dirt particle, dried in oven at 60°C & powdered. 25gms of the plant material was kept in 100ml of methanol for 24hours & then boiled till the volume gets reduced to one–third. The crude extracts were obtained by filtration through Whatman filter no.1 and stored at 4°C (Lin *et al*., 2004).

Collection of pathogenic microbes

Three pure culture of pathogenic bacteria, *Escherichia coli*, *Staphylococcus aureus* & *Pseudomonas* sps., were collected from

Azyme Bioscience Pvt. Ltd. Research Centre, Bangalore. They were mass cultured in nutrient broth, & used for the study of antibacterial activity of plant extracts on them.

Antibacterial study (Agar cup diffusion method)

Antibacterial effect of methanolic leaf (L) and stem (S) extracts were determined by using cup diffusion method on nutrient agar medium (NAM) & Muller Hinton agar (MHA) medium. 200µl of 24 hours of bacterial cultures were aseptically swabed with the help of cotton on the surface of gelled NAM & MHA plates to develop culture lawns. A well of about 6.0mm diameter made aseptically using cork borer in the medum. 50µl of each extract was poured into the wells. A well of negative control was made for extractant (solvent) & positive control was made by placing antibiotic discs of ampicillin & streptomycin on medium. Plates were left for 30minutes for diffusion of extract into the medium & then incubated at 37°C for 24hrs. After incubation, the plates were observed for the zone of inhibition (ZI) of bacterial growth and the diameter of the inhibition zone were measured using geometrc scale.

Results and Discussion

Aqueous & ethanolic extracts of leaves & stems of all plants revealed the presence of secondary metabolites like carbohydrates, alkaloids, steroids, glycosides, flavonoids, terpenoids, quinones, tannins, saponins, proteins and amino acids, phenols, reducing sugars, phlobotannins and, oils & fats. The aqueous extracts have shown the presence of most of secondary metabolites and these were confirmed by ethanolic extraction. However, some secondary metabolites extracted only with aqueous solvent & some with ethanol solvent.

Aqueous extracts have shown more number of phytoconstituents (128), than ethanolic extracts (113) which is to be highlightened (tables 2 & 3)because successive isolation of phytoconstituents from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent (Padmavathy & Mekala, 2013) thus is a positive consideration that phytochemical screening, has shown aqueous extract is found to be more effective than ethanolic extract.

Among five selected plants both aqueous and ethanolic extracts of leaves & stem shown the presence of phytoconstituents like carbohydrates, alkaloids, steroids, glycosides, flavonoids, terpenoids and, oils & fats. But absence of carbohydrates found in leaves of *Mentha piperita* & glycosides in leaves of *Tridax procumbens* of ethanolic extractions. More specifically, the presence of Carbohydrates, Alkaloids, Steroids, Glycosides, Flavonoids, Terpenoids and oils & fats indicate energy source, antimicrobial & anti-diarrheal properties in these plants (Cowan *et al*., 1999; Kumar *et al*., 2010). Presence of Alkaloids has shown anti-microbial, anti-fungal, anti-tumour, cytotoxic, anti-plasmodial, anti-oxidant, anti-mutagenic, anti-genotoxic & hallucinogenic properties. The earliest records of natural products were depicted on clay tablets in cuneiform from Mesopotamia (2600 B.C.) which documented oils from *Cupressus sempervirens* (Cypress) and *Commiphora* species which are still used today to treat coughs, colds and inflammation. Glycoside participates in the biosynthesis & remodulation of glycans, mobilization of energy, defense, symbiosis, signaling, secondary plant metabolism & metabolism of glycolipids (Panter, 2008). Glycoside was found to enhance cardiac conduction, thereby improving the strength

of cardiac contractibility. Presence of Flavonoids in plants has revealed anti-inflammatory, antioxidant, anticancer, antibacterial & antiviral properties (Valsaraj *et al*., 1997; Hollman & Katan, 1999; Harborne & Williams, 2001). Presence of Terpenoids showed anti-inflammatory (Brunaton, 1999) & hemolytic (Heinrich *et al*, 2004) properties. Plant essential oil & fats extracts have been used for many thousand years in food preservation, pharmaceuticals, alternate medicine & normal therapies (Reena *et al*., 2010). Quinones are found absent in both aqueous & ethanolic extracts except in the aqueous extract of stem of *Ocimum tenuiflorum*. Tannins found in both the extracts, but showed negative result in ethanolic extract of leaf of *Ocimum tenuiflorum*. Compared to the other plants, Quinones found absent in ethanolic extraction leaf & stems of all plants, but present only in the aqueous extract of stem of *Ocimum tenuiflorum*. Quinones show a biological, pharmacological activity, and some of them show anti-tumoural activity. They embody some claims in herbal medicine. These applications include purgative, anti-microbial & anti-parasitic, anti-tumour & anti-cardiovascular disease. In 2004, the anti-malarial drug quinine isolated from the bark of *Cinchona succirubra* Pav. Tannins found present in both aqueous & ehanolic extractions of stem & leaves of all plants except in the ethanolic leaf extraction of *Ocimum tenuiflorum*. Tannins show anti-carcinogenic, anti-mutagenic, anti-oxidative, anti-microbial properties, also exert other physiological effects such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level & modulate immunoresponses (Chung, *et al*., 1998). Saponins found present in all but absent only in the ethanolic extraction of stem of *Tridax procumbens.* Presence of saponins in leaf & stem extracts of all plants except *Tridax* plant indicates anti-diarrhael, anti-helminthic, anticancer activities of these plants (Sutar *et al*., 2000; Padmawathy & Mekala 2013). Proteins & amino acids found in all plants except in the aqueous extraction of leaf, and ethanolic & aqueous extractions of leaf & stem respectively of *Tridax procumbens*. Phenols found absent in aqueous extract of stems & ethanolic extraction of leaves of all plant, whereas they are found in aqueous extraction of all leaves except *Tridax procumbens*. Phenols acts as antioxidants also associated with the inhibition of atherosclerosis & cancer (Martinez *et al*., 2000). Reducing sugars have shown positive test with aqueous extraction of both leaves & stems of all plants except in *Mentha piperita* & *Tridax procumbens*. Whereas ethanolic extractions of leaves of *coriandrum*, *Tridax* & *Vinca*, and stems of *Mentha*, *Tridax*, *Ocimum* & *Vinca* are shown positive results & negatives in others. Phlobotannins have shown positive test with aqueous extraction of stems of all plants and leaves of only *Mentha* & *Ocimum*. Whereas, ethanolic extractions of both leaves & stem of all plants shown negative results except in the stem extraction of *Ocimum*.

In addition to the biologically active plant-derived natural products, many other plant derived natural products have served as "lead compounds" for the design, synthesis and development of novel drug compounds (Kinghorn & Balandrin, 1993; Newman *et al*., 2003; Lesney, 2004). In this context, some herb derived natural products have been modified slightly to render them more effective or less toxic in order to produce the so called "semi-synthetic drugs" (Kinghorn & Balandrin, 1993; Kong *et al*., 2003). As an example of this type of strategy, aspirin was developed in 1953 through structural modification of salicylic acid which was identified as the active ingredient in a

number of plants known for their pain relieving qualities (Kong *et al*., 2003; Lesney, 2004).

Secondary metabolites detected by TLC

TLC of leaves & stem extracts of 5 plants revealed different compounds in different parts of the each plant. Detected compounds on TLC for alkaloids in leaves of Coriander, Pudeena, Tridax, Tulsi & Vinca are 3, 5, 5, 4 & 3 respectively. Similarly, stem has 3, 3, 3, 4 & 3 respectively. However, these compounds are different in leaves & stem extracts. This indicates various plant parts have different consequents of phytochemicals. Similarly, the same results have been obtained for Flavonoids, Saponins, and Terpenoids.

TLC results of leaf & stem extracts for biohemicals of all selected plants shown the R*f* values as mentioned in table 4,5,6,7 & Figs.1,2,3,4.

Table.1 Different solvent systems & reagents used for TLC

Secondary Metabolites	Solvent systems	Ratio	Spraying reagents
Alkaloid	Methanol: ammonium hydroxide	(17:3)	Mayer's reagent
Flavonoid	n-butanol: glacial acetic acid: water	(4:1:5)	UV- Light
Saponin	Chloroform: glacial acetic acid: methanol	(6:2:1:1)	Iodine vapours
Terpenoid	Benzene : ethyl acetate	(5:95)	10% H_2So_4

Table.2 Phytochemicals detected in aqueous extracts of leaves & stems.

S.No.	Phytochemicals	Test performed	Aqueous extracts									
			Leaves					Stems				
			L1	L2	L3	L4	L5	S1	S2	S3	S4	S5
a)	Carbohydrates	Molisch's Test	+	+	+	+	+	+	+	+	+	+
b)	Alkaloids	Mayer's Test	+	+	+	+	+	+	+	+	+	+
c)	Steroids	Ring Test	+	+	+	+	+	+	+	+	+	+
d)	Glycosides	Salkowski's Test	+	+	+	+	+	+	+	+	+	+
e)	Flavonoids	Lead acetate Test	+	+	+	+	+	+	+	+	+	+
f)	Terpenoids	Ring Test	+	+	+	+	+	+	+	+	+	+
g)	Quinones	Conc.HCl Test	-	-	-	-	-	-	-	-	+	-
h)	Tannins	Lead acetate Test	+	+	+	+	+	+	+	+	+	+
i)	Saponins	Froathing Test	+	+	+	+	+	+	+	+	+	+
j)	Proteins & amino acids	Ninhydrin Test, Millon's Test	+	+	-	+	+	+	+	+	+	+
k)	Phenols	Ferric chloride Test	+	+	-	+	+	-	-	-	-	-
l)	Reducing sugars	Fehling's Test	+	-	-	+	+	+	+	+	+	+
m)	Phlobotannins	Boiling Test	-	+	-	+	-	+	+	+	+	+
n)	Oils & Fats	Spot Test	+	+	+	+	+	+	+	+	+	+

(+) present, **(-)** absent

Table.3 Phytochemicals detected in ethanolic extracts of leaves & stem.

S.No	Phytochemicals	Test performed	Ethanolic extracts									
			LEAF SAMPLES					STEM SAMPLES				
			L1	L2	L3	L4	L5	S1	S2	S3	S4	S5
a)	Carbohyderates	Molisch's Test	+	-	+	+	+	+	+	+	+	+
b)	Alkaloids	Mayer's Test	+	+	+	+	+	+	+	+	+	+
c)	Steroids	Ring Test	+	+	+	+	+	+	+	+	+	+
d)	Glycosides	Salkowski's Test	+	+	-	+	+	+	+	+	+	+
e)	Flavonoids	Leadacetate Test	+	+	+	+	+	+	+	+	+	+
f)	Terpenoids	Ring Test	+	+	+	+	+	+	+	+	+	+
g)	Quinones	Conc.HCl Test	-	-	-	-	-	-	-	-	-	-
h)	Tannins	Leadacetate Test	+	+	+	-	+	+	+	+	+	+
i)	Saponins	Froathing Test	+	+	+	+	+	+	+	-	+	+
j)	Proteins & amino acids	Ninhydrin Test, Millon's Test	+ / +	+ / +	+ / -	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +	+ / +
k)	Phenols	Ferric chloride Test	-	-	-	-	-	-	-	-	-	-
l)	Reducing sugars	Fehlings Test	+	-	+	-	+	-	+	+	+	+
m)	Phlobotannins	Boiling Test	-	-	-	-	-	-	-	-	+	-
n)	Oils & Fats	Spot Test	+	+	+	+	+	+	+	+	+	+

(+) present, **(-)** absent

Table.4 Rf values detected for identification of alkaloids

Sample	Plant part	R*f* value	Plant part	R*f* value
Coriander	L1	0.24, 0.76, 0.84	S1	0.88,0.94,0.99
Pudeena	L2	0.16, 0.39, 0.46, 0.52, 0.59	S2	0.70, 0.83, 0.94
Tridax	L3	0.16, 0.37, 0.47, 0.54, 0.59	S3	0.74, 0.83, 0.90
Tulsi	L4	0.13, 0.26, 0.33, 0.44	S4	0.20, 0.66, 0.83, 0.91
Vinca	L5	0.27, 0.34, 0.84	S5	0.68, 0.86, 0.93

Table.5 Rf values detected for identification of flavonoids.

Sample	Plant part	R*f* value	Plant part	R*f* value
Coriander	L1	0.91	S1	0.15, 0.93, 0.98
Pudeena	L2	0.95, 0.94	S2	0.56, 0.89, 0.93
Tridax	L3	0.91, 0.86	S3	0.73, 0.86, 0.91
Tulsi	L4	0.95, 0.99	S4	0.95, 0.99
Vinca	L5	0.28, 0.65, 0.90	S5	0.21, 0.41, 0.58, 0.92, 0.94

Table.6 Rf values detected for identification of saponins.

Sample	Plant part	R*f* value	Plant part	R*f* value
Coriander	L1	0.89, 0.99	S1	0.65, 0.93
Pudeena	L2	0.62, 0.90	S2	0.84, 0.88, 0.89
Tridax	L3	0.10, 0.31, 0.83	S3	0.28, 0.81
Tulsi	L4	0.15, 0.5, 0.80	S4	0.84, 0.88, 0.95
Vinca	L5	0.16, 0.48, 0.6, 0.9	S5	0.85, 0,86, 0.93

Table.7 Rf values detected for identification of terpenoids.

Sample	Plant part	R*f* value	Plant part	R*f* value
Coriander	L1	0.04, 0.06, 0.11, 0.70, 0.81, 0.96	S1	0.07, 0.91, 0.96
Pudeena	L2	0.03, 0.05, 0.09, 0.24, 0.78, 0.85, 0.93	S2	0.08, 0.90,0.99
Tridax	L3	0.03, 0.06, 0.22, 0.36, 0.76, 0.85, 0.95	S3	0.06, 0.28, 0.90, 0.98
Tulsi	L4	0.04, 0.07, 0.08, 0.27, 0.76, 0.86, 0.94	S4	0.31, 0.92, 0.98
Vinca	L5	0.04, 0.08, 0.11, 0.15, 0.56, 0.88, 0.96	S5	0.06, 0.15, 0.91, 0.96

L-leaf, S-stem.

Table.8 Growth inhibition (mm) of three bacteria by leaf extracts & comparison with positive and negative controls on NAM & MHA

Bacteria	***E.coli***						***Staphylococcus aureus***						***Pseudomonas***					
Media	**NAM**			**MHA**			**NAM**			**MHA**			**NAM**			**MHA**		
Hours	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
L1	0.2	0.3	0.5	0.2	0.3	0.4	0.0	0.0	0.0	0.3	0.5	0.7	0.3	0.4	0.4	0.2	0.4	0.6
L2	0.2	0.3	0.4	0.1	0.3	0.4	0.0	0.0	0.0	0.2	0.5	0.6	0.2	0.3	0.4	0.2	0.3	0.3
L3	0.1	0.3	0.4	0.1	0.3	0.4	0.0	0.0	0.0	0.1	0.2	0.3	0.2	0.3	0.4	0.2	0.3	0.3
L4	0.2	0.4	0.5	0.1	0.2	0.3	0.0	0.0	0.0	0.4	0.5	0.7	0.2	0.3	0.4	0.2	0.3	0.3
L5	0.4	0.7	1.0	0.2	0.4	0.5	0.0	0.0	0.0	0.2	0.4	0.7	0.4	0.5	0.5	0.2	0.3	0.3
Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amp^r+	0.8	1.0	1.2	0.2	0.5	0.8	0.4	0.6	0.8	0.9	1.3	1.5	0.6	0.8	0.8	0.5	0.7	0.9
Strep^r+	1.2	1.4	1.6	0.8	1.1	1.6	0.9	1.1	1.3	0.8	1.0	1.4	0.8	1.0	1.1	1.2	1.3	1.3

Table.9 Growth inhibiton (in mm) of three bacteria by stem extracts & comparison with positive and negative controls on NAM & MHA

Bacteria	***E.coli***						***Staphylococcus aureus***						***Pseudomonas***					
Media	**NAM**			**MHA**			**NAM**			**MHA**			**NAM**			**MHA**		
Hours	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**	**24**	**48**	**72**
S1	0.1	0.2	0.3	0.1	0.2	0.3	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.3	0.2	0.2	0.2
S2	0.0	0.1	0.1	0.1	0.2	0.2	0.0	0.0	0.0	0.0	0.1	0.1	0.2	0.3	0.3	0.2	0.2	0.2
S3	0.1	0.3	0.4	0.2	0.3	0.4	0.0	0.0	0.0	0.2	0.2	0.2	0.2	0.3	0.4	0.1	0.3	0.4
S4	0.2	0.3	0.3	0.0	0.1	0.1	0.0	0.0	0.0	0.1	0.3	0.4	0.2	0.3	0.4	0.2	0.3	0.4
S5	0.1	0.2	0.3	0.1	0.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.3	0.2	0.3	0.3
Methanol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Amp^r+	0.5	0.5	0.6	1.5	1.6	1.8	1.1	1.3	1.5	1.6	1.8	1.9	0.4	0.6	0.7	0.7	0.9	1.1
Strep^r+	0.8	0.9	1.3	1.4	1.6	1.8	0.8	1.2	1.5	1.2	1.6	1.7	0.9	1.2	1.7	0.4	0.5	0.8

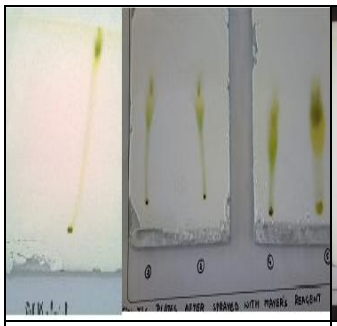

Fig.1a:Alkaloids in leaves extract

Fig.1b:Alkaloids in stem extract

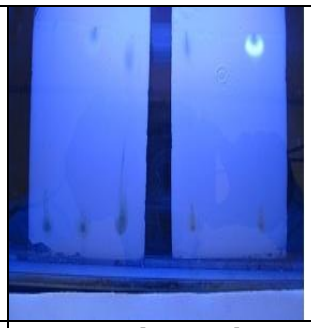

Fig.2a: Flavanoids in leaves extract

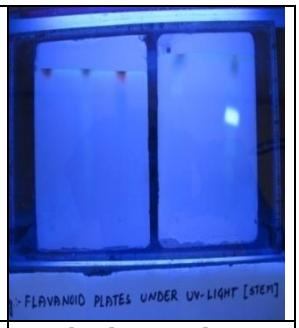

Fig.2b:Flavanoids in stem extract

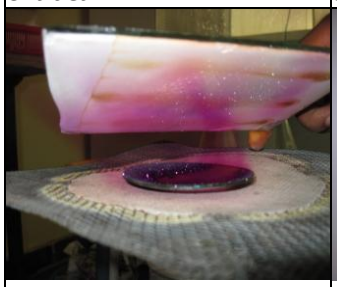

Fig.3a: Saponins in leaves extract

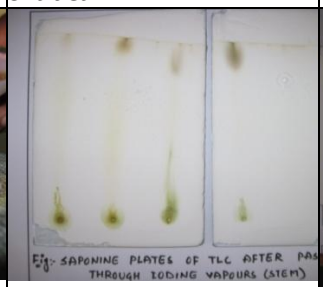

Fig.3b:Saponins in stem extract

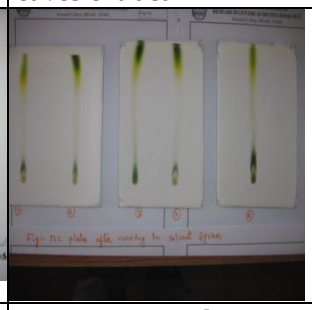

Fig.4a:Terpenoids in leaves extract

Fig.4b:Terpenoids in stem extract

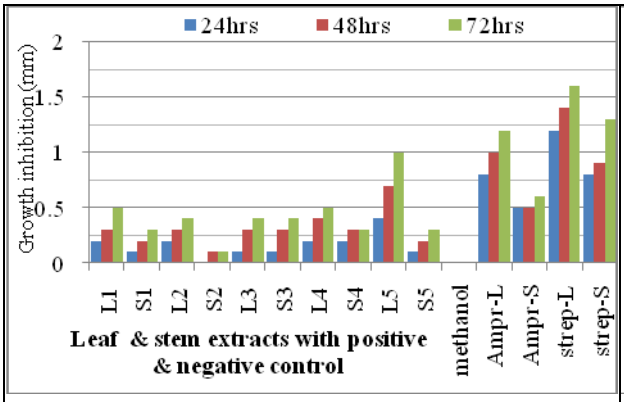

Fig.5a: Growth inhibition of *E.coli* by leaf & stem extracts of 5-plants on NAM.

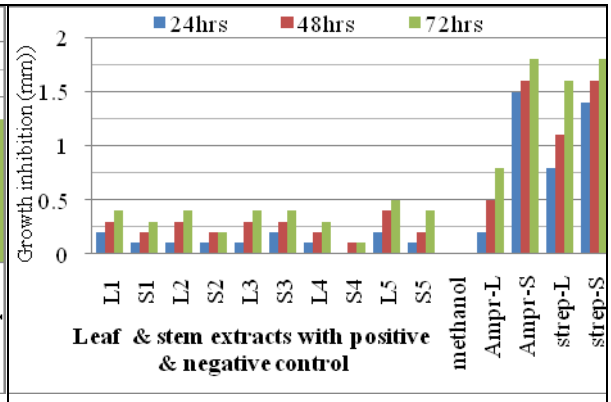

Fig.5b: Growth inhibition of *E.coli* by leaf & stem extracts of 5-plants on MHA.

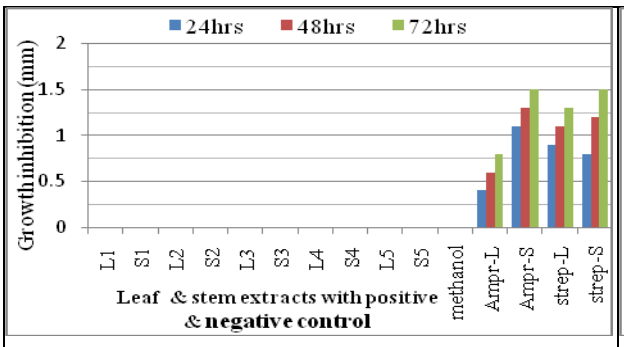

Fig.6a: Growth inhibition of *S.aureus* by leaf & stem extracts of 5-plants on NAM.

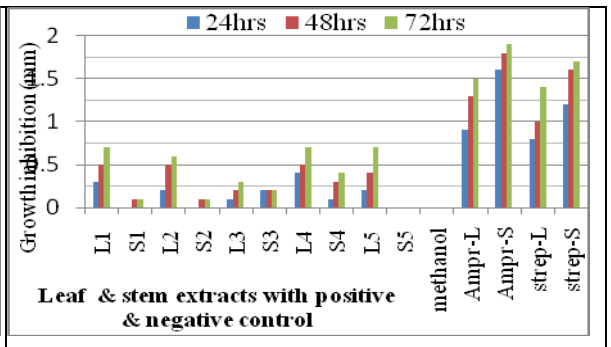

Fig.6b: Growth inhibition of *S.aureus* by leaf & stem extracts of 5-plants on MHA.

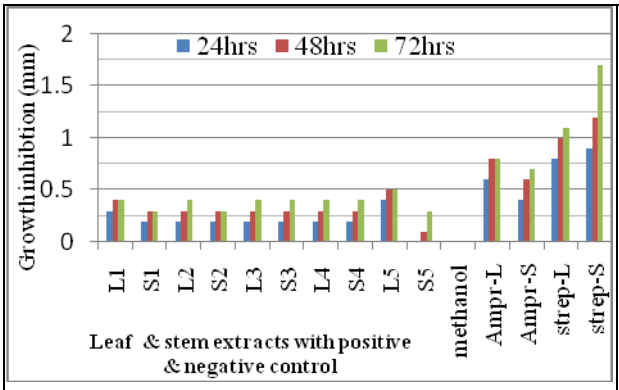

Fig.7a: Growth inhibition of *Pseudomonas sps.* by leaf & stem extracts of 5-plants on NAM.

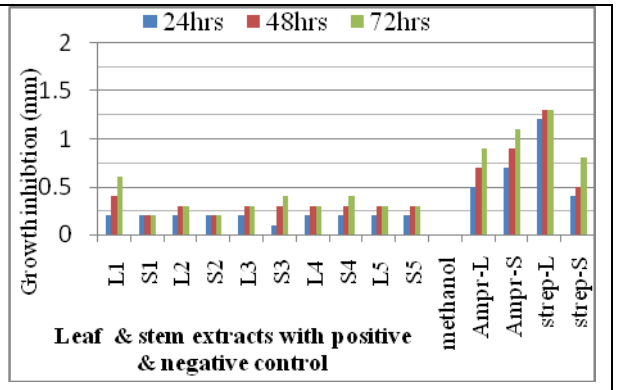

Fig.7b: Growth inhibition of *Pseudomonas sps.* by leaf & stem extracts of 5-plants on MHA.

Antibacterial effects of various extracts

Effects on *E.coli* (figs.5a&b)

The methanolic extracts of all plants have shown antibacterial effects on the growth of the cultures of *E.coli* in both NAM & MHA. Further, the antibiotic effect of leaf extract has more than extracts of stem. In addition, it was also found that their effects with all extracts found more on NAM than on MHA. Whereas, its results were vice-versa with both +ve controls.

Higher effect on NAM by plant extracts than on MHA may be due to higher diffusion rate of extract into the NAM, than in MHA as the solid disc of +ve control has variation with these effects. The antibiotic effect of all extracts is not because of individual secondary metabolites but is the combined effect of all metabolites, as mentioned in tables 8 & 9.

Effects on *Staphylococcus aureus* (figs.6a&b)

All plant extracts have shown antibacterial effect with the bacterial culture on MHA except with the extract of S_5. Whereas, these bacteria not grown on the NAM. However, both +ve controls have significant antimicrobial effect with the cultures on both MHA & NAM.

The plant extracts showed higher diffusion in MHA & exhibited growth inhibition, but NAM might not supported for the multiplication of bacteria, hence zone of inhibition was not found on it. Significant zone of growth inhibition was observed with +ve control only on MHA as it was seen in previous results with *E.coli*.

Effects on *Pseudomonas* sps. (Figs.7a & b)

All plant extracts have shown antibacterial effect with the cultures of *pseudomonas* sps.on both NAM & MHA. Further, the antibiotic effect of leaf extract is more than extracts of stem. In addition, it was also found that the zone of growth inhibition was found more on NAM than MHA. Whereas, significant growth inhibition was found with streptomycin but not with ampicillin control. Higher effect on NAM by plant extracts than on MHA may be due to diffusion rate of extract into the NAM may be less than in MHA, as the solid disc of +ve control has no significant variation with these effects.

Thus, selected plant sps are potentially good source of traditional medicines &

therapeutics. Presence of bioactive compounds like Alkaloids, Steroids, Glycosides, Flavonoids, Terpenoids, Quinones, Tannins, Saponins, Phenols, & Phlobotannins in leaves & stems of five plants can be used for the treatment of different ailments like Malaria, Diarrhoea, Skin burn, Antimicrobial agents etc. Antimicrobial activity showed the highest zone of growth inhibition by leaf extract of *Vinca roseus* (1.0 cm) on *E.coli* bacteria.

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