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Screening of Antimicrobial Activity of Indian Medicinal Plants

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Abstract

Crude extracts from seeds viz., Hydnocarpus wightiana, Nyctanthes arbor-tristis, Wrightia tinctoria, Alangium lamarckii and Carum copticum were prepared using the solvents ethanol, methanol, acetone and aqueous. The extracts were screened for their antibacterial activity against four bacterial pathogens and phytochemical analysis were also carried out. The tested bacterial strains were Escherichia coli, Salmonella typhi, Proteus mirabilis and Klebsiella pneumoniae. Among the extracts methanol extract of all the seeds exhibited higher activity followed by ethanol, aqueous and acetone. Among the test organisms Klebsiella pneumoniae and Proteus mirabilis showed higher sensitivity towards all the seed extracts followed by Escherichia coli and Salmonella typhi. Carum copticum was found to be highly effective comparatively to others. The antibacterial potential of the extracts were found to be dose dependent. These activities of the seeds were due to the presence of various secondary metabolites.

Key words: Antibacterial activity, Bacterial pathogens, Secondary metabolites

Introduction

Medicinal plants form a large group of important flora. Plants provide basic raw materials for the indigenous pharmaceutical industries such as medicinal, cosmetic, perfumery and food etc (Cowan, 1999). 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 (Rathis *et al*., 2005). 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 (Ramasamy and Charles Manoharan, 2004).

The use of the leaves, flowers, stem, seed berries and roots of the plants are known to prevent, relieve and treat illness. They also play vital role as an antimicrobial agent (Deshpande *et al*., 2005). From 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 (Tambekar and Saratkar, 2005). Today science has isolated the medicinal properties of a large number of botanicals and their healing 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 of variety of organisms and multiple drug resistant organisms pose serious threats to treat infectious diseases. Hence plant derived antimicrobial have received considerable attention in recent years.

As per an estimate of WHO, about 80% of the population in developing countries relay on traditional medicine for their primary health care. Moreover 20% of prescribed drugs presently are formulated from the herbal plants only (Gulliermo Delgado and Victor Navarro, 1999). Plants cells are highly sophisticated chemical factors where a large variety of chemical compound are manufactured with great precision and ease from simple raw materials at normal temperature and pressure. Plants are thus a variety of chemicals. It is estimated that there are 2,50,000 to 5,00,000 species of plants on earth. A relatively small percentage (1-10%) of these are used as foods by both humans and other animal species. It is possible that even more are used for medicinal purpose (Orawan Runangsamboom *et al*., 1999).

The antimicrobial properties of plants have been investigated by number of researches word wide, especially in Latin America. In Argentia, a research tested 122 known plant species used for therapeutic treatments (Pia Morela *et al*., 2000). It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococcus aureus*, ten inhibited the growth of *Escherichia coli* and four inhibited *Aspergillus niger*.

Although extremely effective, antibiotics are able to induce resistance in bacteria. For >50 years, bacterial resistance has been the main factor for the increase of morbidity, mortality and healthcare costs of bacterial infections (Hideyo Yamaguchi *et al*., 2001). In vivo tests of each plant extract against malarial and bacterial infection would have been time consuming and expensive without obtaining the desired knowledge. It was therefore decided to screen each plant extract was found to inhibit the test organism, it was then investigated further by more comprehensive in vitro and in vivo tests (Towers *et al*., 2008).

Use of plant as a source of medicine have been inherited and is an important component of the health care system in India public, academic and government interest in traditional medicines is growing exponentially due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine. Medicinal plants were used in Ayurveda for treating diseases, they are used as antibacterial, antifungal and antiviral properties.

Materials and Methods

Collection of plant materials

The fresh and healthy seeds of five plants *Hydnocarpus wightiana, Nyctanthes arbor-tristis, Wrightia tinctoria, Alangium laamrckii, Carum copticum* were collected from Chidambaram district. The plant material like seed were washed thoroughly with tap water and then with sterilized distilled water for the removal of dust and sand particles. The seeds were shade dried for few days and then powdered. This was used as raw material for the extraction of antimicrobial compounds against the microbes.

Culture used

Microorganisms chosen were isolated from the clinical specimens that came for culture and sensitivity testing done in the laboratory of Department of Microbiology, Annamalai University. The cultures identified were *E.coli, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae*.

Preparation of plant extracts

The preparations of different plant extract was done though modified method (Perumal Samy *et al*., 2002).

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Aqueous extraction methods

The shade dried seed material were used for the aqueous extraction procedure, about 5 gm of seed powder were weighed and mixed with sterile distilled water (1:1 w/v) which was incubated for two days. Later filtered the slurry through filter paper and centrifuged the filtrate at 5000 rpm for 10 min. Collected the supernatant and used it for its antimicrobial activity test by dissolving the supernatant in required concentration with dimethyl sulfoxide (DMSO).

Solvent extraction methods

The shade dried seed materials were used for the solvent (methanol, ethanol and acetone) extraction procedure, about 5 gm of seed powder were weighed and mixed with respective solvent individually (1:3 w/v), which was incubated for two days. After the incubation period the slurry was filtered through whatman no.1 filter paper in a beaker and allowed it for evaporation. The residue was dissolved with dimethyl sulfoxide (DMSO) with different concentrations and checked it for its antimicrobial activity.

Antimicrobial susceptibility test

Disc diffusion method was adopted for evaluation of antimicrobial activity of five different medicinal seeds. Muller Hinton agar was prepared and autoclaved at 151b pressure for 20 minutes and cooled at 45°C. The cooled media was poured on to sterile petriplates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using steril swab. The disc impreginated with respective seed extract at different concentration (200, 400, 600 mg/ml) individually were placed on the four corners of each petridishes, control disc was also placed. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

Phytochemical screening

The seed extract of *Hydnocarpus wightiana, Nyctanthes arbor-tristis, Alangium lamarckii, Carum copticum* were analyzed for the presence of saponins, Tannins, Alkaloids, Flavonoids, Triterpenoids, Steroids, Anthraquinones, Glycosides, Coumarin, Gum, Starch and Protein (Kumar *et al.*, 2009).

Test for Saponins

The extract was diluted with 20 ml of distilled water and it was agitated in a graudated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

Test for Tannins

Five ml of the extract and a few drops of 1% lead acetate were added yellow precipitate was formed, which indicates the presence of tannins.

Test for Alkaloids

Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Drangendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

Test for Flavonoids

To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

Test for Triterpenoids

Ten mg of the extract was dissolved in 1 ml of chloroform to which 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid formation of reddish violet colour indicates the presence of triterpenoids.

Test for Steroids

About 100 mg of the extract was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a low layer. A reddish brown colour at the interface is indicate for the presence of steroids.

Test for Anthraquinones

Five ml of the extract solution was hydrolysed with diluted concentrated sulphuric acid to which1 ml of dilute ammonia was added. Rose pink colouration suggest the positive response for anthraquinones.

Test for Glycosides

About 100 mg of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring obtained indicate the presence of glycosides.

Test for Coumarin

To about 100 mg of the extract a few drops of 10% sodium hydroxide was added yellow colour formation indicate the presence of coumarin.

Test for Gum

To the seed powder drops of water was added swelling of seeds / forms adhesive indicate the presence of gum.

Test for Starch

To the extract drops of iodine solution was added formation of blue colour indicate the presence of starch.

Test for Protein

To the extract drops of picric acid was added formation of yellow colour indicate the presence of starch.

Results

The antimicrobial activity of crude seed extract of *Hydnocarpus wightiana, Nyctanthes arbor-tristis, Wrightia tinctoria, Alangium lamarckii* and *Carum copticum* were studied in different concentration (200 mg/ml, 400 mg/ml, 600 mg/ml).

The *Hydnocarpus wightiana* seed extract was extracted using different solvent (Methanol, Ethanol, Acetone and Aqueous), their antimicrobial activity was studied on *Escherichia coli, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae*. The result showed that among the extracts, methanol extract showed high inhibitory effect, at (200, 400, 600 mg/ml) the zone size measured was (-, 7 and 9 mm) for *Proteus mirabilis*, (-, 7 and 11 mm) for *Klebsiella pneumoniae* and for *E.coli* the zone size was (8 mm, 11mm, 12 mm) and (10 mm, 11mm, 12mm) for *Salmonella typhi*. In *Nyctanthes arbor-tristis* seed extract, Methanol extract showed higher activity on the microbes used when compared to other solvent extract. The zone size measured was (12, 13 and 14 mm) for *Proteus mirabilis*, (15, 16 and 18 mm) for *Klebsiella pneumoniae*, (7,9 and 10 mm) for *E.coli* and (9, 11 and 12 mm) for *Salmonella typhi*.

Table 1: Antimicrobial activity of crude seed extract of *Hydnocarpus wightiana* in comparison to different extraction solvent at different concentration (200 mg/ml, 400 mg/ml and 600 mg/ml)

Serial No	Solvents used for extraction	Microorganism	Zone of inhibition in millimeter			
			Concentration in mg/ml			
			Control	200	400	600
1.	Aqueous extract	*Escherichia coli*	-	8	11	13
		Salmonella typhi	-	10	11	12
		Proteus mirabilis	-	-	7	9
		Klebsiella pneumoniae	-	-	7	11
2.	Methanol extract	*Escherichia coli*	-	11	13	14
		Salmonella typhi	-	12	14	15
		Proteus mirabilis	-	8	11	13
		Klebsiella pneumoniae	-	11	12	13
3.	Ethanol extract	*Escherichia coli*	-	11	13	14
		Salmonella typhi	-	12	13	15
		Proteus mirabilis	-	11	14	16
		Klebsiella pneumoniae	-	12	13	14
4.	Acetone extract	*Escherichia coli*	-	12	14	15
		Salmonella typhi	-	13	13	15
		Proteus mirabilis	-	10	12	13
		Klebsiella pneumoniae	-	13	13	15

(-) - No growth

Graph 1: Antimicrobial activity of crude seed extract of *Hydnocarpus wightiana* in comparison to different extraction solvent at 600 mg/ml

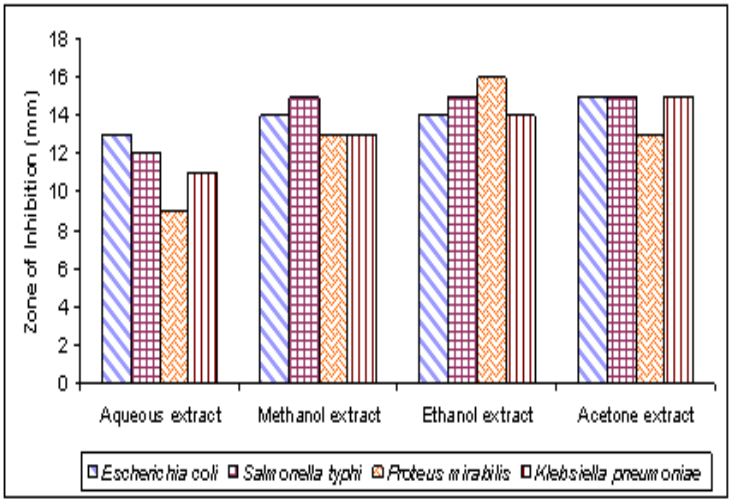

Table 2: Antimicrobial activity of crude seed extract of *Nyctanthes arbor-tristis* in comparison to different extraction solvent at different concentration (200 mg/ml, 400 mg/ml and 600 mg/ml)

Serial No	Solvents used for extraction	Microorganism	Zone of inhibition in millimeter			
			Concentration in mg/ml			
			Control	200	400	600
1.	Aqueous extract	*Escherichia coli*	-	6	7	9
		Salmonella typhi	-	7	9	11
		Proteus mirabilis	-	13	14	15
		Klebsiella pneumoniae	-	14	15	16
2.	Methanol extract	*Escherichia coli*	-	7	9	10
		Salmonella typhi	-	9	11	12
		Proteus mirabilis	-	12	13	14
		Klebsiella pneumoniae	-	15	16	18
3.	Ethanol extract	*Escherichia coli*	-	5	7	8
		Salmonella typhi	-	5	7	7
		Proteus mirabilis	-	10	11	13
		Klebsiella pneumoniae	-	12	14	15
4.	Acetone extract	*Escherichia coli*	-	5	6	7
		Salmonella typhi	-	6	7	7
		Proteus mirabilis	-	9	10	13
		Klebsiella pneumoniae	-	11	12	12

(-) - no growth

Graph 2: Antimicrobial activity of crude seed extract of *Nyctanthes arbor-tristis* in comparison to different extraction solvent at 600 mg/ml

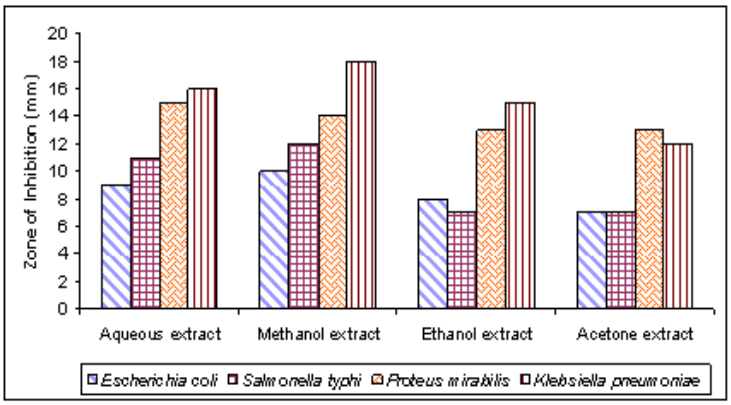

Table 3: Antimicrobial activity of crude seed extract of *Wrightia tinctoria* in comparison to different extraction solvent at different concentration (200 mg/ml, 400 mg/ml and 600 mg/ml)

Serial No	Solvents used for extraction	Microorganism	Zone of inhibition in millimeter			
			Concentration in mg/ml			
			Control	200	400	600
1.	Aqueous extract	*Escherichia coli*	-	4	5	6
		Salmonella typhi	-	4	7	7
		Proteus mirablis	-	8	10	11
		Klebsiella pneumoniae	-	10	12	15
2.	Methanol extract	*Escherichia coli*	-	7	7	8
		Salmonella typhi	-	5	7	8
		Proteus mirabilis	-	11	14	16
		Klebsiella pneumoniae	-	14	15	18
3.	Ethanol extract	*Escherichia coli*	-	4	5	7
		Salmonella typhi	-	5	7	10
		Proteus mirabilis	-	10	12	14
		Klebsiella pneumoniae	-	11	13	14
4.	Acetone extract	*Escherichia coli*	-	5	6	7
		Salmonella typhi	-	5	6	6
		Proteus mirabilis	-	9	10	12
		Klebsiella pneumoniae	-	12	13	14

(-) - no growth

Graph 3: Antimicrobial activity of crude seed extract of *Wrightia tinctoria* in comparison to different extraction solvent at 600 mg/ml.

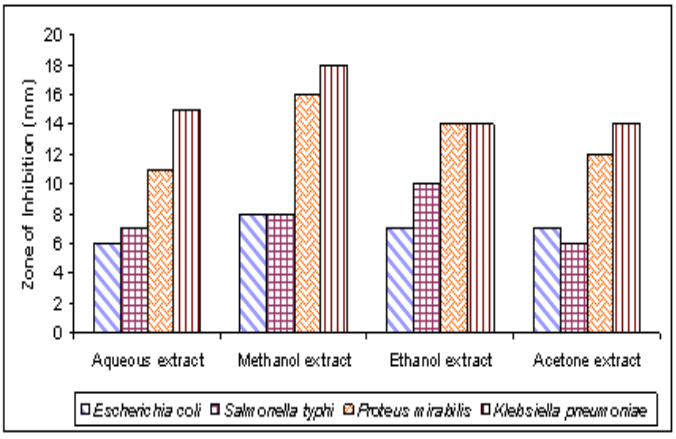

Table 4: Antimicrobial activity of crude seed extract of *Alangium lamarckii* in comparison to different extraction solvent at different concentration (200 mg/ml, 400 mg/ml and 600 mg/ml)

Serial No	Solvents used for extraction	Microorganism	Zone of inhibition in millimeter			
			Concentration in mg/ml			
			Control	200	400	600
1.	Aqueous extract	*Escherichia coli*	-	4	6	7
		Salmonella typhi	-	4	5	6
		Proteus mirabilis	-	7	9	10
		Klebsiella pneumoniae	-	8	11	12
2.	Methanol extract	*Escherichia coli*	-	7	7	9
		Salmonella typhi	-	7	9	10
		Proteus mirabilis	-	9	12	14
		Klebsiella pneumoniae	-	13	15	19
3.	Ethanol extract	*Escherichia coli*	-	5	7	9
		Salmonella typhi	-	9	6	7
		Proteus mirabilis	-	11	10	11
		Klebsiella pneumoniae	-	5	12	13
4.	Acetone extract	*Escherichia coli*	-	5	7	8
		Salmonella typhi	-	4	5	6
		Proteus mirabilis	-	10	11	11
		Klebsiella pneumoniae	-	11	12	12

(-) - no growth

Graph 4: Antimicrobial activity of crude seed extract of *Alangium lamarckii* in comparison to different extraction solvent at 600 mg/ml

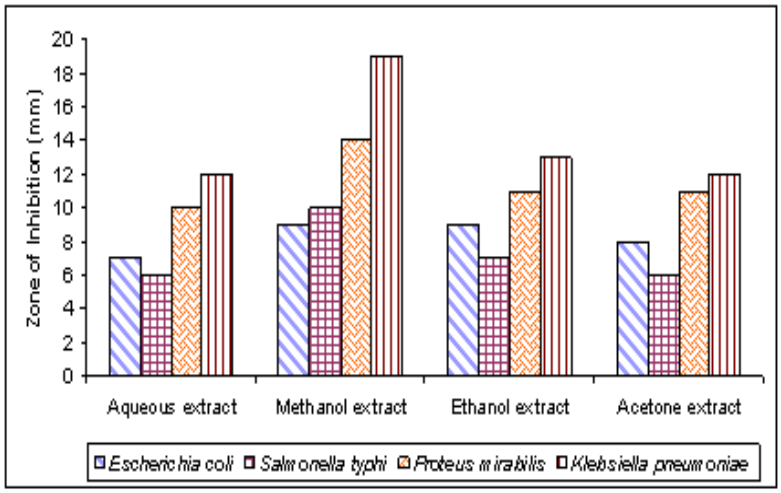

Table 5: Antimicrobial activity of crude seed extract of *Carum copticum* in comparison to different extraction solvent at different concentration (200 mg/ml, 400 mg/ml and 600 mg/ml)

Serial No	Solvents used for extraction	Microorganism	Zone of inhibition in millimeter			
			Concentration in mg/ml			
			Control	200	400	600
1.	Aqueous extract	*Escherichia coli*	-	6	7	9
		Salmonella typhi	-	7	9	10
		Proteus mirabilis	-	8	10	11
		Klebsiella pneumoniae	-	11	12	14
2.	Methanol extract	*Escherichia coli*	-	10	10	11
		Salmonella typhi	-	8	10	11
		Proteus mirabilis	-	11	13	17
		Klebsiella pneumoniae	-	16	18	22
3.	Ethanol extract	*Escherichia coli*	-	7	8	9
		Salmonella typhi	-	8	9	11
		Proteus mirabilis	-	10	11	13
		Klebsiella pneumoniae	-	11	11	13
4.	Acetone extract	*Escherichia coli*	-	7	8	9
		Salmonella typhi	-	7	7	10
		Proteus mirabilis	-	8	9	10
		Klebsiella pneumoniae	-	11	13	14

(-) - no growth

Gaphy 5: Antimicrobial activity of crude seed extract of Carum copticum in comparison to different extraction solvent at 600 mg/ml

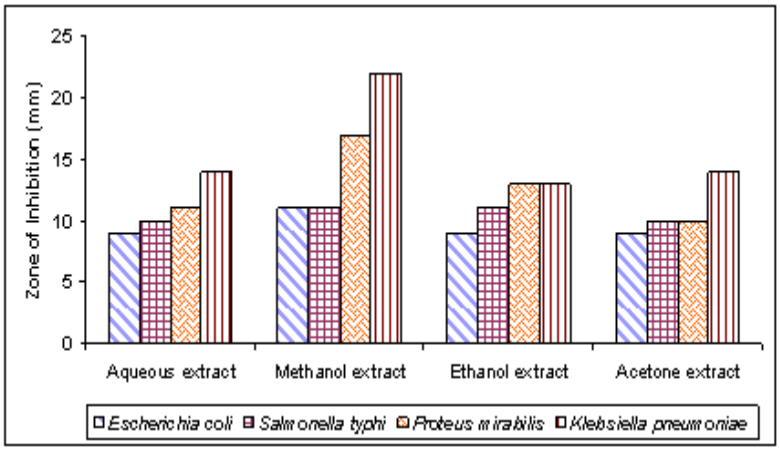

Table 7: Phytochemical composition of seed

Serial No	Secondary metabolite	*Hydnocarpus wightiana*	*Nyctanthes arbor-tristis*	*Wrightia tinctoria*	*Alangium lamarckii*	*Carum copticum*
1.	Saponins	-	-	+	-	+
2.	Tannins	+	-	-	+	+
3.	Alkaloids	-	+	-	+	-
4.	Falvonoid	-	+	+	-	-
5.	Triterpenoids	-	+	-	+	-
6.	Steroids	-	-	+	+	+
7.	Glycosides	+	+	+	+	+
8.	Anthraquinones	+	+	-	-	-
9.	Coumarin	+	+	+	-	-
10.	Gum	-	-	+	-	+
11.	Starch	-	-	-	-	-
12.	Protein	+	+	+	+	+

(-) – Negative (+) - Positive

Wrightia tinctoria seed extract was also extracted using different solvent, compared to other solvent Methanol extract showed higher activity. The zone size measured was (14, 15 and 18 mm) for *Klebsiella pneumonia,* (11, 14 and 16 mm) for *Proteus mirablis*, (7, 7 and 8 mm) for *E. coli* and (5,7 and 8 mm) for *Salmonella typhi*. Similarly in *Alangium lamarckii* and *Carum copticum* seed extract, methanol extract showed higher activity and the zone size measured was (13, 15 and 19 mm) in *Klebsiella pneumoniae* for *A. lamarckii* and (16, 18 and 22 mm) in *Klebsilla pneumoniae* for *Carum copticum*, for *Proteus mirabilis* the zone size was measured as (9, 12 and 14 mm) for *A. lamarckii* and (11, 13 and 17 mm) for *Carum copticum,* for *E. coli* the sensitivity was measured as (7, 7 and 9 mm) for *A. lamarckii* and (10, 10 and 11 mm) for *Carum copticum*, for *Salmonella typhi* the sensitivity was measured as (7, 9 and 10 mm) for *A. lamarckii* and (8, 10 and 11 mm) for *Carum copticum*.

The results show that among the five seeds used *Carum copticum* was found to be more effective against the microbes used than the four plant seed. This study also shows the presence of various secondary metabolites in various seeds (Table 7).

Discussion

Plants are known to contain innumerable biologically active compounds. Essential oils occur in sixty families and many of them have been reported to possess biological activity such as antifungal, antibacterial and insect repellent. These are easily extracted from the plant tissue without any changes in active compounds in composition (Nunel Absar *et al*., 2003).

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the worlds population (Masatake Niwa *et al*., 2003). There are about 45,000 plant species in India with capacity to produce a large number of organic chemicals concentrated hotspot in the region of Eastern Himalays, of high structural diversity.

In the present work methanolic extract of *Carum copticum* showed higher activity to the tested bacteria such as *Klebsiella pneumoniae, Proteus mirabilis, Escherichia coli* and *Salmonella typhi.* The antibacterial activity of *Hydnocarpus uightiana, Nyctanthes arbor-tristis, Wrightia tinctoria, Alangium lamarckii* showed more or less equal zone of inhibition or slightly greater against some pathogens when compared to each other.

Crude extracts prepared using the solvents ethanol, acetone and aqueous from the seeds too showed more or less equal zone of inhibition or slightly greater against the pathogens. But all results showed *Carum copticum* to have higher activity followed by *Alangium lamarckii, Nyctanthes arbor-tristis, Wrightia tinctoria* and *Hydnocarpus wightiana* towards the microbes used. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The results of phytochemicals in the present investigation showed that all the seeds more (or) less contain same components like Saponin, Tannins, Steroids, Glycoside, Phytosterol and Protein (Table 7). The seeds can also have various medicinal values such as anti-inflammatory, anti-diabetic and analgesic activities and also for central nervous system activity. Even though this is only a preliminary study of the occurrence of certain properties in seeds an

in-depth study will provide a good concrete base of all the phytochemicals functions mentioned (Aiyelaagbe and Paul, 2009).

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