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Screening for antimicrobial activity of some medicinal plants.

R. P. Hongalamath and E. B. Sedamkar
Department. of Botany, KLE'S G. H. College, Haveri. (Karnataka), India
Corresponding author: E. B. Sedamkar (ebsedamkargkl@gmail.com)

Abstract: *During present study the antibacterial activity of the petroleum ether extracts of Vitex negundo, Duranta repens, Piper nigrum and Acorus calamus were analysed. The antibacterial potential of these extracts were tested on Streptococcus mutans and Pseudomonas aeruginosa. The extracts have shown considerable inhibitory effect on test organisms Various combinations of the four plant extracts were studied for their synergistic activity and the results got are promising. The combined plant extracts have shown better antibacterial activity than compared to individual activity of plant extracts.*

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I. Introduction:

Nature has been a source of medicinal agents for thousand of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world.(Mahesh et al,2008).

Bioactive products of plant origin being less persistent in environment, easily biodegradable safe to mammals and other no-target organisms have therefore become the focus of attention today. The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs.(Cathrine et al,2011).

In the present work four selected plants were screened for potential antibacterial activity.

1. Vitex negundo : (Family:Lamiaceae)

Vitex negundo is a large aromatic shrub. It has typical five foliated leaf pattern. Flowers are pedunculate, branched, cymes, opposite along the quadrangular rachis of a large terminal often compound pyramidal panicle. Its leaves and seeds are widely used externally for rheumatism and inflammations (Tandon & Gupta,2004) of joints and are also reported to have insecticidal properties. Internally decoction of its leaves is taken as diuretic, expectorant, vermifuge tonic and febrifuge. Its essential oil is found to be useful for sloughing wounds and ulcers. The leaves of Vitex negundo are reported to possess pesticidal, antifungal and antibacterial properties.

2. Duranta repens : (Family Lamiaceae)

Duranta repens is commonly called as Golden dew drops. It is a small genus consists of shrubs or trees which are mostly armed with axillary thorns and have simple opposite decussate leaves. Flowers are small, mostly blue, purple or white and bracteates. The aqueous extract of leaves and fruits also shows insecticidal and antifeedant properties against Aedes aegypti and Attagenus piceus. It is a poisonous plant and has caused deaths of children. Saponins in the fruits and foliage cause gastroentric irritation, drowsiness, fever, vomiting.

3. **Piper nigrum [Black Pepper-** Family Piperaceae.]

Black pepper is the dried, unripe fruit of Piper nigrum a climbing plant. The plant produces a pendulous spike of sessile fruits, which are collected and dried. The volatile oil of black pepper has high degree of stimulating and carminative properties of the volatile oils, causing a reflex flow of saliva with increased secretion of gastric juice. In sufficient doses, the pepper dilates the superficial vessels of the skin, causing a feeling of warmth followed by diaphoresis and reduction in temperature. It is diuretic & antibacterial(Singh et.al,2005)

4. **Acorus calamus : (**Family Acoroceae)

Both the leaves and rhizomes are apparently psychoactive. Studies have shown that it is mutagenic in bacteria. It was used as an anesthetic for toothache and headache. It is used in the stomach and bowel because it stimulates the salivary glands and production of stomach juices, helping to counter acidity and cease heartburn

and dyspepsia. Acorus calamus is antirheumatic and analgesic. The essential oil is anticonvulsant antiveratrinic and antiarrhythmic. In ayurveda it is valued as a rejuvenator for the brain and nervous system.

II. Materials and Methods:

1. Microbial strains:

The pure cultures of the required microbial strains were procured from MTCC, Chandigarh, India. The organisms procured are;

a] Pseudomonas aeruginosa [MTCC 0741]

b] Streptococcus mutans [MTCC 890]

2. Selection of part of plants;

Here some of the medicinally important plants are selected for screening of antimicardial activity, and the parts used are as follows;

a] Leaves of Duranta repens and Vitex negundo

b] Seeds of Piper nigrum

c] Rhizome of Acorus calamus

3. Chemicals :

Petroleum ether is used as a solvent for the extraction of plant material. Nutrient agar and brain heart infusion agar for bacterial cultivation, standard antibiotics like Ampicillin, Ciprofloxacin and Erythromycin were purchased from HI media laboratories India Ltd.

The plant material was ground to a fine powder before extraction. The powder [50g] was then extracted with petroleum ether solvent, using soxhlet apparatus by continuous heat extraction for 36 hours. The extracts obtained were concentrated to dryness by evaporating the solvent under reduced pressure. The concentration thus obtained was dissolved in DMSO (Dimethyl sulfoxide) in such a way that the final concentration of the extract would be 20mg/ml of DMSO.

III. Disc diffusion method :

The invitro antibacterial activity of the petroleum ether extracts of plants were carried out by disc diffusion method. (Hanan.H & Eman.A,2012). Actively growing log phase cultures of S.mutans and P.aeruginosa were mixed in brain heart infusion agar medium and nutrient agar medium respectively and plated. The sterile discs were placed on each part of the plates and the discs were loaded with appropriate volume of plant extract to get the concentration of 100 µg, 200 µg, 300 µg and 400 µg and the plates were incubated at 37º`C for 24 hours. Similarly the synergistic action was also tested on each of the organism by making known concentration of combined extracts of plant in the ratio 1:1. The combinations of plant extracts made were;

1. V.negundo & A.calamus
2. V.negundo & P.nigrum
3. D.repens & A.calamus
4. D. repens & P.nigrum.

The diameter of zone of growth inhibition was measured in mm. The effects were compared with that of the standard antibiotic ampicillin and ciprofloxacin for P.aeruginosa and erythromycin for S.mutans and DMSO alone served as control.

IV. Results and Discussion:

The zone of inhibition was measured for all the four plant extracts and the results are depicted in Table-1. It was found that Gram +ve S.mutans was more susceptible than Gram –ve bacteria, but less efficient than that of standard antibiotics, Ciprofloxacin, Ampicillin and Erythromycin.

The flower oil of V.nigundo was found to be active against P.aeruginosa (Khokra et al.,2008). Aqueous chloroform and methanol extracts of V.nigundo were tested,against P.aeruginosa. Of these only aqueous extract failed to show inhibitory effect(Loganathan et al.,2005.)The results obtained from our studies indicate that the petroleum ether extracts of leaves of V.nigundo shown inhibitory activity against P.aeruginosa (Vasinauskiene et.al,2006)and S.mutans at a concentration of 300µg.The inhibitory effect was found to be more on P.aeruginosa than S.mutans at this concentration.From our studies it was observed that petroleum ether extracts of D.repens exhibited considerable inhibitory activity against S.mutans at a concentration of 200µg, P.aeruginosa at 300µg. This shows that S.mutans is more susceptible than P.aeruginosa.The ethyl acetate extract of P.nigrum was tested against Gram +ve and Gram –ve bacteria.It is reported that Gram –ve bacteria are not inhibited by ethyl acetate extract of P.nigrum at concentrations of ≤ 1000 µg/ml(Yunck et al.,2001).From our studies it was observed that petroleum ether extracts of P.nigrum did not show inhibitory effect on P.aeruginosa, but it showed inhibitory effect on S.mutans at a concentration of 300µg and 400µg. This shows

that S.mutans is more susceptible than P.aeruginosa.Findings of our study are similar to those reported by Yunck et al.,(2001).

From our studies P.aeruginosa was found to be more susceptible to A.calamus at concentrations of 200 µg, 300µg and 400µg and same results were observed in case of S.mutans. Similarly the zone of inhibition was measured for synergistic activity of the plant extracts and the results are depicted in Table-2. The combined activity of A.calamus and P.nigrum with V.negundo and D.repens in 1:0.5 ratios did not show any inhibitory effect; where as antibacterial effect was observed in 1:1 ratio, with the combined extracts.The extract of P.nigrum did not show any inhibitory effect on tested organisms, but the combination of P.nigrum with V.negundo and D.repens showed considerable inhibitory effect.The standard antibiotic ampicillin did not show any inhibitory effect on P.aeruginosa,whereas the plant extracts except P.nigrum have shown considerable inhibitory effect on P.aeruginosa.Ciprofloxacin has shown inhibitory effect on P.aeruginosa with an inhibition zone of 15mm which is similar to the inhibitory effect shown by combined plant extracts of D.repens+P.nigrum and V.negundo+A.calamus with an inhibition zone of 15mm and 16mm respectively. Erythromycin was found to be more effective against S.mutans than compared to any of the plant extracts tested, which showed an inhibition zone of 27mm.

Table - 1. Individual Activity Of Plant Extracts OnS.mutans & P.aeruginosa

Sl. No.	Plant Extract	Concen tration of Stock Solution.	Total volume loaded on disc.	Respective amount of extract present.	Diameter of inhibition zone [mm]	
					S.mutans.	P.aeruginosa
1.	V.negundo	20 mg/ml	5 µl	100 µg	0	0
			10 µl	200 µg	0	0
			15 µl	300 µg	7 mm	8 mm
			20 µl	400 µg	9 mm	10 mm
2.	D.repens	20 mg/ml	5 µl	100 µg	0	0
			10 µl	200 µg	7 mm	0
			15 µl	300 µg	9 mm	9 mm
			20 µl	400 µg	10 mm	11 mm
3.	P.nigrum	20 mg/ml	5 µl	100 µg	0	0
			10 µl	200 µg	0	0
			15 µl	300 µg	7 mm	0
			20 µl	400 µg	10 mm	0
4.	A.calamus	20 mg/ml	5 µl	100 µg	0	0
			10 µl	200 µg	7 mm	8 mm
			15 µl	300 µg	8 mm	10 mm
			20 µl	400 µg	10 mm	11 mm
5.	DMSO	20 mg/ml	5 µl	-	0	0
			10 µl	-	0	0
			15 µl	-	0	0
			20 µl	-	0	0

Table - 2. Synergistic Activity Of Plant Extracts On S.mutans & P.aeruginosa

S.no	Plant extract combination	Conc.of each extract	Total volume loaded on disc	Respective amount of each plant extract in the disc		Diameter of inihibition zone (mm)	
				V.negundo	P.nigrum	S.mutans	P.aeruginosa
1	V.negundo + P.nigrum	40mg/ml	10µl	200µg	200µg	8mm	8mm
			15µl	300µg	300µg	10mm	10mm
			20µl	400µg	400µg	11mm	11mm
			25µl	500µg	500µg	11mm	12mm
				V.negundo	A.calamus		
2	V.negundo +A.calamus	40mg/ml	10µl	200µg	200µg	10mm	10mm
			15µl	300µg	300µg	11mm	12mm
			20µl	400µg	400µg	11mm	13mm
			25µl	500µg	500µg	12mm	16mm
				D.repens	P.nigrum		
3	D.repens+ P.nigrum	40mg/ml	10µl	200µg	200µg	10mm	10mm
			15µl	300µg	300µg	11mm	11mm
			20µl	400µg	400µg	12mm	14mm
			25µl	500µg	500µg	13mm	15mm
				D.repens	A.calamus		
4	D.repens+ A.calamus	40mg/ml	10µl	200µg	200µg	8mm	11mm
			15µl	300µg	300µg	9mm	12mm
			20µl	400µg	400µg	10mm	12mm
			25µl	500µg	500µg	12mm	13mm

Table - 3. Antibacterial Activity Of Standard Antibiotics On S.mutans & P.aeruginosa

Sl. No.	Name of the standard Antibiotic	Concentration	Organism tested	Diameter of inhibition zone [mm]
1.	Ampicillin	25 mg/disc	P.aeruginosa	O
2.	Ciprofloxacin	10 mg/disc	P.aeruginosa	15 mm
3.	Erythromycin	15 mg/disc	S.mutans	27 mm

V. Conclusion :

Present study shows that plant extracts have great potential as antimicrobial compounds against micro-organisms. The plant extracts which are used have shown considerable antibacterial activity and so these plants can be used to discover bioactive natural products that may serve as lead for the development of new pharmaceuticals that address hitherto unmet theropeutic needs. The millenarian use of these plants in folk medicine suggests that they represent on economic and safe alternative to treat infectious diseases.

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