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ANTIMICROBIAL ACTIVITY AND WOUND HEALING EFFICIENCY OF HERBS AND HONEY

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ABSTRACT

In ancient period, they practiced medicine with plants and used the plants as a source of medicine. Plants are an important component of the health care system in India. Indian medicinal plants are the essence of Ayurveda and Ayurvedic treatments and they produce miraculous effects. Traditional medicine practices were carried out with natural resources to cure many dreadful diseases. Herbal medicine or phytomedicine refers to the use of any plant's seeds, berries, roots, leaves, bark or flowers for medicinal purposes. The present investigation was carried out using natural resources of medicinal plants and Honey to check for the healing capacity of wound and inhibit the infectious pathogens such as *Staphylococcus aureus, Pseudomonas, Klebisella spp; Escherichia coli*. The plants were extracted with solvents like methanol, ethanol and also subjected to direct extraction. The results of this study showed that all the natural resources have the wound healing properties but the extent of healing depended on the concentration.

KEYWORDS: Honey, Medicinal Plants, Pathogens and Solvents

INTRODUCTION

Plants are the primary natural source of medicine. Medicinal plants are considered to be very rich sources of secondary metabolites and oils which are of therapeutic importance. The important advantages of medicinal plants in various treatments are their safety, besides being less expensive, efficiency and availability throughout the world. Use of plants as a source of medicinal value is a very old concept. Chinese were the first to use plants as therapeutics before 4000-5000B.C. In India use of plants as a medicine appeared in Rig-Veda which has been written 3500-1600B.C. Properties of plants as a source of medicine were studied in detail in Ayurveda which is considered to be the foundation of all the medicinal science. Microorganisms develop resistances against various antibiotics and due to this an immense clinical problems develops in treatment of infectious diseases. All such issues can be overcome by the secondary metabolites of medicinal plants which possess many therapeutic properties against pathogens. In the present study antimicrobial efficiency of *Aloe vera, Tridax procumbens*, Ramathulsi, Honey and citrus peel were investigated against wound pathogens.

MATERIALS AND METHODS

Sample Collection

Medicinal plants (*Aloe Vera*, *Tridax procumbens*, Ramathulsi, Orange peel) and Honey was collected from different natural habitats of the country. *Aloe Vera, Tridax procumbens*, Thulsi were collected from the local areas of Palakkad, Kerala. Honey samples were collected from the local beekeepers of different areas of Palakkad. Citrus peels were collected from the local fruit juice shops of Palakkad.

Extraction Method

Aloe vera

Solvent Extraction

The collected plant gel was freeze dried and then grinded to get crude extract. The crude extract is filtered through Whatmann filter paper. Then 1gm gel extract was mixed with 5ml of ethanol and kept under shaker for overnight [1]. After overnight incubation, the mixture was filtered through Whatmann No.1 paper and it was evaporated to dryness at room temperature. After evaporation, the pellet was resuspended with 0.5ml of Dimethylsulphoxide (DMSO) using micro syringe and recollected for further use. Plant powder residue left after ethanol extraction was sequentially extracted with benzene, methanol and chloroform. The re-suspended solvents were used for antimicrobial assay.

Direct Extraction

Mature, healthy and fresh leaves of *Aloe Vera* with an approximate length of 0.762-0.914m, were collected and washed with fresh water. The thick epidermis was selectively removed. The inner colorless, mucilaginous pulp was homogenized and centrifuged at 4^{o}C for 15 minutes to remove the fibers. The supernatant was transferred to a sterile glass vial for further test.

Tridax Procumbens

Solvent Extraction

Different parts of *T. procumbens* (stem, leaf, and flowers) were collected; shade dried, finely powdered using a blender and subjected to extraction [2]. Hundred grams of each finely powdered sample was extracted using Soxhlet apparatus with 80% hot methanol (500 ml) on a water bath for 24 hours and filtered. Filtrate was re-extracted successively. The powered samples were extracted with Benzene, Chloroform respectively using the same procedure. The extracts were stored at 4°C and were re-suspended in their respective solvents to get 10 mg/ml for antimicrobial assay.

Direct Extraction

Fresh leaves of *Tridax procumbens* were collected and separated into stem, leaves and flowers. The separated parts are homogenized by using blender. The extracts were filtered through Whatmann No: 1 filter paper. Then filtered samples collected in a clean glass vial and stored at 4^{o}C before use.

Ramathulsi

Solvent Extraction

Thoroughly washed plants are separated into leaves, flowers and stem. Selected plant parts were separately shade dried, finely powdered using a blender and subjected to solvent extraction method. Using chemicals such as benzene, methanol and chloroform, 2g of each finely powdered sample were soaked in separate conical flask having 10ml of solvents each. It left for 24 hours so that alkaloids, terpenoids and other constituents get dissolved. The solvent extract was filtered using Whatmann no. 1 filter paper, again filtered through sodium sulphate in order to remove the traces of moisture. Phytochemicals screening of the plant extract was carried out as per the methods and tests given by Dey and Raman (1957) and was stored at 4^{o}C for antimicrobial assay.

Direct Extraction

Freshly collected Thulsi leaves were grinded using motor and pestle. Sample filtered using Whatmann No: 1 filter paper. Collected samples were stored at 4^{o}c for antimicrobial activity.

Honey

Direct Extraction

Honey samples were collected from the local beekeepers of different areas of Palakkad. The samples were stored in half liter plastic containers duly labeled with numbers, names and date of collection. Unwanted material such as wax sticks, dead bees and particles of combs were removed by straining the samples through cheesecloth. Then physicochemical properties were analyzed. Determination of pH of acidity, moisture, ash, electrical conductivity, sucrose and total sugars from honey samples were according to the method of AOAC (2000) and EU Council (2002). Total protein contents were determined by Bradford (1976) and AOAC (2000).

Citrus Peel

Solvent Extraction

Citrus peels were collected from the local fruits juice shops. After collection, the peels were shade dried at room temperature (32^{o}C-35^{o}C). Peels were coarsely powdered using an electric blender. 10g of powdered plant materials were soaked in 100ml of methanol, benzene, chloroform and water [1]. It was left for 24 hours. The extracts were filtered through Whatmann No: 1 filter paper. The filtered extracts were transferred to glass vials and kept at 4^{o}C before use.

Direct Extraction

10g of powdered plant material was soaked in 100ml of distilled water at ambient temperature for 24 hours under shaking condition at 130 rpm. The extract was then filtered using Whatmann filter paper No:1. Each extract was transferred to glass vials and kept at 4^{o}C before use.

COLLECTION OF PUS FROM WOUND SAMPLES

The study was carried out in the Microbiology Laboratory of Nehru Arts and Science College, Coimbatore and the wound samples were collected from patients of Government Hospital, Coimbatore with the help of a physician. Pus from an abscess was collected and a piece of tissue specimen from the wound was collected before an antiseptic dressing and special care was taken to avoid contaminating the specimen with commensal organism from the skin. After taking the samples in the sterilized containers, routine microbial analysis were performed and all the organisms present in wound sample are confirmed by cultural and biochemical characteristics [4].

ANTIMICROBIAL ACTIVITY

The organisms isolated from wound samples *[Staphylococcus aureus, Pseudomonas, Klebisella spp; Escherichia coli*] were grown and maintained on Muller-Hinton agar medium, while *Candida albicans* was maintained on Sabouraud Dextrose agar medium.

Disc diffusion assay [5] was performed for screening bacterial and fungal inoculums (inoculums size 1×10^{8} cfu/ml for bacteria and 1×10^{7}cells/ml for fungi) on MH agar and SD agar plates respectively. Sterile filters paper discs (Whatmann No:1) of 6mm in diameter were impregnated with 100µl of each of the extract to give a final concentration of

1mg/disc and left to dry in vacuum so as to remove residual solvent, which might interfere with the determination. Extract discs were then placed on the seeded agar plates. Each extracts was tested in triplicate with Streptomycin (1mg/disc) and Terbinafine (1mg/disc) as standard for bacteria and fungi respectively. The plates were kept at 4^{o}C for 1hour for diffusion of extract, thereafter they were incubated at 37^{o}C for bacteria (24hour) and 27^{o}C for fungi (48hour).

RESULTS

The wound samples collected from hospital were processed in the laboratory and based on the microscopic characteristics and biochemical features organisms were identified as *Staphylococcus aureus*, *Streptococcus sp*, *Klebsiella sp*, *Pseudomonas* sp, *Proteus* sp, *Escherichia coli and Candida albicans* (Table -1). The sample was mixed in fluid thioglycollate broth and immediately a drop was transferred onto the petriplates containing three different types of medium Viz Mac Conkey Agar medium, Nutrient Agar medium, Blood Agar medium and Mannitol Salt Agar medium (Table -2).

The effects of different concentration of different plant extracts against test organisms were studied. An inhibition zone >14mm was considered as a high antibacterial activity. The result showed that increased concentration of extracts revealed increased zone of inhibition. Among the five plant extracts *Tridax procumbens* and *Ramathulsi* showed better activity against all the test organisms isolated from wound sample. The solvent extracts (Methanol, benzene, chloroform) showed higher activity against all test organisms than the direct extract (Table.3-11).

Table 1: Microscopic, Cultural Characteristics and Biochemical Features of Test Organisms

Sl. No	Tests	*S. Aureus*	*Streptococcus*	*Klebsiella*	*Pseudomonas*	*E. coli*	*Proteus*
1	Gram staining	+ve, cocci	+ve, cocci	-ve, Rod	-ve, Rod	-ve, Rod	-ve, Rod
2	Motility	-ve	-ve	-ve	+ve	-ve	-ve
3	Catalase	+ve	-ve	+ve	+ve	-ve	+ve
4	Oxidase	-ve	-ve	-ve	+ve	-ve	-ve
5	Indole	-ve	+ve	-ve	-ve	+ve	+ve
6	Methyl-red	+ve	-ve	-ve	+ve	+ve	+ve
7	Voges-proskaeur	+ve	-ve	+ve	-ve	-ve	-ve
8	Citrate	-ve	-ve	+ve	+ve	-ve	+ve
9	Urease	+ve	-ve	+ve	+ve	-ve	+ve

Table 2: Cultural Characteristics

Sl. No	Name of the Pathogen	McConkey Agar		Mannitol Salt Agar		Blood-Agar	
		Colour of the Medium	Colour of the Colony	Colour of the Medium	Colour of the Colony	Colour of the Medium	Colour of the Colony
1	*S.aureus*	-	-	Red	Golden yellow	Red	White
2	*Streptococcus*	-	-	-	-	Red	White
3	*Pseudomonas*	-	-	White	Pale green	-	-
4	*Klebsiella*	Pink	Pink	-	-	-	-
5	*Proteus*	Pink	Pale pink	-	-	-	-
6	*E.coli*	Pink	White	-	-	-	-

Table 3: Antimicrobial Activity of Different Concentration of Solvent Extracts of Aloe Vera

Organisms	Solvent Extracts	Zone of Inhibition(Mm)		
		20µl	40µl	60µl
S. aureus	E_1	8	10	12
	E_2	2	5	8
	E_3	1	4	8

Streptococcus	E_1	2	4	8
	E_2	-	-	-
	E_3	-	-	-
Pseudomonas	E_1	2	4	7
	E_2	-	-	-
	E_3	-	-	-
Klebsiella	E_1	2	2	4
	E_2	-	2	2
	E_3	-	-	-
Proteus	E_1	-	-	-
	E_2	-	-	-
	E_3	-	-	-
E.coli	E_1	2	4	10
	E_2	-	2	6
	E_3	-	2	4

E_1 = METHANOL E_2 = BENZENE E_3 = CHLOROFOAM

Table 4: Antimicrobial Activity of Different Concentration of Direct Extracts of Aloevera

Organisms	Zone of Inhibition (mm)		
	20µl	40µl	60µl
S.aureus	2	6	10
Streptococcus	2	4	6
Pseudomonas	1	1.5	2
Klebsiella	-	-	0.5
Proteus	-	-	-
E.coli	2.5	3	4

Table 5: Antimicrobial Activity of Different Concentration of Solvent Extracts of Tridax Procumbens

Plant	Plant Parts	Solvent Extract	Test Organism																	
			S.Aureus			*Streptococcus*			*Pseudomonas*			*Klebsiella*			*Proteus*			*E.coli*		
			20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Tridax procumbens	Leaf	E_1	-	10	12	-	-	5	10	12	14	10	11	13	-	-	10	10	12	14
		E_2	11	13	14	2	4	8	-	10	11	-	10	11	-	-	10	10	11	12
		E_3	2	6	9	2	6	8	-	-	-	-	-	3	5	8	10	10	12	14
	Stem	E_1	10	14	16	-	-	-	8	13	14	10	11	13	-	-	-	10	12	13
		E_2	11	13	14	-	-	-	-	10	11	-	10	11	-	-	10	10	11	12
		E_3	12	14	15	-	-	-	-	-	-	5	8	10	8	10	14	4	5	7
	Flower	E_1	15	20	22	-	2	4	10	11	12	10	11	12	10	11	13	10	12	14
		E_2	11	13	14	-	-	8	-	10	11	-	10	14	11	12	13	10	12	14
		E_3	8	10	13	-	-	-	-	10	12	-	2	4	2	5	7	2	4	6

E_1 = Methanol **E_2** = benzene **E_3** = Chloroform

Table 6: Antimicrobial Activity of Different Concentration of Direct Extracts of Tridax Procumbens

Plant	Plant Part	Extract Concentration in µg/ml	Test Organisms					
			S.Aureus	*Streptococcus*	*Pseudomonas*	*Klebsiella*	*Proteus*	*E.coli*
Tridax procumbens	Leaf	**20**	4	-	-	1	-	2
		40	8	-	2	2	2	6
		60	10	-	3	8	4	8
	Stem	**20**	10	2	8	2	-	1
		40	10	4	11	4	4	4
		60	11	6	12	6	8	6
	Flower	**20**	-	-	-	10	-	1
		40	6	-	-	11	-	2
		60	9	-	2	13	7	5

Table 7: Antimicrobial Activity of Different Concentration of Solvent Extracts of Ramathulsi Plant

Plant	Plant Part	Solvent Extracts	Test organisms																	
			S.Aureus			*Streptococcus*			*Pseudomonas*			*Klebsiella*			*Proteus*			*E.coli*		
			20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Tridax procumbens	Stem	E_1	2	4	8	-	-	4	1	4	7	-	-	4	-	-	2	2	4	6
		E_2	2	6	10	-	2	4	-	-	4	-	-	-	-	-	-	2	3	7
		E_3	4	8	12	-	-	-	2	2	4	2	2	4	2	4	6	2	4	8
	Leaf	E_1	2	4	8	-	-	-	-	-	-	-	4	8	2	4	6	2	4	6
		E_2	2	8	10	-	-	-	-	-	-	-	2	4	-	-	2	-	4	8
		E_3	2	8	12	-	-	-	-	-	-	-	-	2	-	-	4	2	4	10
	Flow-er	E_1	-	-	-	-	-	4	-	2	6	-	-	-	-	-	-	4	8	10
		E_2	-	-	-	-	-	2	-	2	6	-	-	-	-	1	2	2	8	12
		E_3	-	-	-	-	2	4	2	4	8	-	-	-	1	2	4	4	6	8

E_1= METHANOL E_2 = BENZENE E_3 = CHLOROFOAM

Table 8: Antimicrobial Activity of Different Concentration of Direct Extract of Ramathulsi

Plant	Plant Part	Test organisms																	
		S.Aureus			*Pseudomonas*			*Streptococcus*			*Klebsiella*			*Proteus*			*E.Coli*		
		20	40	60	20	40	60	20	40	60	20	40	60	20	40	60	20	40	60
Ramathulsi	Leaf	2	4	8	-	-	4	-	-	-	-	-	2	-	2	4	2	4	6
	Stem	2	2	4	-	-	-	-	2	4	-	2	4	1	1.5	2	2	2.5	3
	Flower	1	2.5	3	-	-	1	-	1	2	-	-	-	-	-	-	1	2	3

Table 9: Antimicrobial Activity of Different Concentration of Solvent Extracts of Citrus Peel

Bacteria	ZONE OF INHIBITION(mm)								
	Acetone			Ethanol			Benzene		
	20	40	60	20	40	60	20	40	60
S.aureus	4	6	10	2	4	8	0.4	2	7
Streptococcus	2	8	10	-	2	9	2	7	8
Pseudomonas	-	-	7	4	6	10	-	-	-
Klebsiella	4	8	12	2	5	10	-	2	8
Proteus	-	-	-	-	-	-	-	-	-
E.coli	4	6	12	4	5	11	2	5	8

Table 10: Antimicrobial Activity of Citrus Peel Extract Obtained by Direct Method

Bacteria	Zone of Inhibition(mm)		
	20µl	40µl	60µl
S.aureus	1	6	8
Streptococcus	-	-	7
Pseudomonas	-	-	-
Klebsiella	-	3	9
Proteus	-	-	-
E.coli	3	6	10

Table 11: Antimicrobial Activity of Different Concentration of Honey

Clinical Isolates	Zone of Inhibition		
	20	40	60
E.coli	1	15	18
S.aureus	1	15	22
Proteus	0.2	0.2	1
Klebsiella	0.2	0.5	1
Streptococcus	14	18	18
Pseudomonas	0.2	0.3	0.5

DISCUSSIONS

All medicinal plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combination of secondary metabolites such as alkaloids, steroids, tannins and phenol compounds present in the plant. In plants, these compounds are synthesized and deposited in specific parts or in all parts of the plant. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct ([7] [8]). The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites.

In the present study the most common organisms isolated from wound sample are *Staphylococcus aureus, Streptococcus, Pseudomonas, Klebsiella, Proteus* and *E.coli*. Among these organisms, *S.aureus* is the predominant organism. The isolates were subjected to antibiotic sensitivity by disc diffusion method using solvent and direct extracts to find out most effective extracts for the treatment of Pyogenic infection.

Antimicrobial activity of different concentration of solvent extracts of *Aloe vera* were treated against test organisms, among which methanol and benzene extracts exhibited the highest zone of inhibition. *S.aureus* and *E.coli* shows 10mm (60µl) and 8mm (60µl) zone of inhibition respectively. Direct extracts of *Aloe vera* exhibited high activity against *S.aureus*.

The Methanolic leaves extracts (13mm) and Chloroform flower extracts (12mm) of *Tridax procumbens* at concentration of 60µl shows high zone of inhibition against *S.aureus.* Methanolic leaf extracts at concentration of 60µl exhibited zone of inhibition of 14mm against *E.coli.* Direct extracts of *Tridax procumbens* stem (11mm) shows maximum zone of inhibition against *S.aureus* and leaf (10mm) at concentration of 60µl shows inhibition against *E.coli.*

The chloroform leaf and stem extracts of Ramathulsi showed maximum zone of inhibition against *S.aureus* (12mm) at 60µl and *E.coli* (8mm) at 60µl.These might be due to the presence of Phytochemicals such as alkaloids, tannins, saponin, steroids, terpenoids and flavonoids in leaf of Ramathulsi. [9]. Flavonoids are known to be synthesized by plants in response to microbial infection. They have been found to be effective antimicrobial substances *in vitro* against a wide array of infections agents [10] in direct method leaf extracts shows high zone of inhibition against *S.aureus* and *E.coli*

Antimicrobial efficiency of three different solvents (Acetone, Benzene, Ethanol) were screened against six pathogenic bacteria *Staphylococcus aureus, Streptococcus, E.coli, Proteus, Pseudomonas, Klebsiella*. The highest antimicrobial potential was exhibited by acetone extract of citrus peel followed by ethanol extracts of citrus peel.

The antimicrobial action of honey was treated against S.*aureus* and other organisms isolated from infected wounds, among which S.*aureus* shows maximum zone of inhibition (22mm) at concentration of 60µl.

CONCLUSIONS

Based on the results it is observed that methanol extracts of *Aloe vera, Tridax procumbens* (leaf), Ramathulsi (leaf), Acetone citrus peel extracts and Honey was found to be active against *S.aureus* and *E.coli*.

Direct extracts of *Aloe vera*, *Tridax procumbens* (stem), Ramathulsi (leaf), Citrus peel, Honey extracts were active against *S.aureus*. *Tridax procumbens* (leaf), Ramathulsi (leaf), Citrus peel and Honey were active against *E.coli.* Hence, the present investigation clearly indicates that the use of medicine for treating wound infectious and the importance of the

traditional medicinal plants for the discovery of new bioactive substances. Further studies may be necessary to elevate the specific photoactive compounds present in *Aloevera*, *Tridax procumbens*, Ramathulsi, Honey and Citrus peel against wound infectious microorganisms.

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