A COMPARATIVE STUDY ON ANTIOXIDANT AND ANTICANCER PROPERTIES OF MORINDA SP, GYMNEMA SP, EUPHORBIA SP, PTEROCARPUS SP AND ITS ANTIMICROBIAL ACTIVITY AGAINST WOUND INFECTION

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ABSTRACT

Skin wound healing occurs through a process of connectivity tissuse repair involving interactions between specialized cells. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia with impaired carbohydrates, fat,and protein medabolisum. Traditional medicines derived from medicinal plants are used by about 60% of the world's populations.This review focuses on Indian herbal drugs and plants used in the treatment of diabetics and it's secondary complications herbal formulations are preferred due to lesser side effect and low cost. A listof medicinal plants with proven antidiabetic and related beneficial effects and herbal drugs used in treatment of diabetics is complied.These includes ***Morinda citrifolia , Euphorbia hirta, Gymnema sylvestre , Pterocarpus santalinus*** **.**one of the ethological factors implicated in the development of diabetic and it's complications is the damage induced by free radicals and hence an antidiabetics compound with antioxidant properties would be more beneficial.

Keywords: antidiabetic compound, antioxidant, herbal drugs

1.INTRODUCTION

Medicinal plants are rich source of antimicrobial agents plants are used medicinally in different countries and are the sources of potential and powerful drugs.The substances that can either inhabits the growth of pathogens or kill them and have no or least toxicity to host cells are considered candidates for developed new antibacterial drugs. In recent years antimicrobial properties of medicinal plants are being increasingly reports from different parts of world .Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as microorganism . one such resources is folk medicines.Systematic screening of them may result in the discovery of novel effective compounds.

India processes a rich biodiversity of the medicinal plants that were still not explored completetly.The need for the novel pharmaceutical products out from the plant has attained great interest in the presence biomedical researches due to potent source of natural antioxidant ***Morinda citrifolia , Euphorbia hirta , Gymnema sylvestre , Pterocarpus santalinus*** is the medicinal plants that shown different type of pharmacological activity like antimicrobial ,antioxidant ,anticancer.The aim of this study was to evaluvate the wound healing activity of extracts from the leaves in normal The four different plants was found to possess a large number of phytochemicals. Alkaloids are found in higher percentage due to that is secure wound healing and antidiabetic properties **(Anurag singh and Pramod kumar singh,2014)**

Microorganism and medicinal plants are rich sources of secondary metabolites which are potential source of useful drugs and others useful bio reactive project. Scientific experiment on the antimicrobial properties of plant compounds were first documented in the 19th century. Microorganisms are closely associated with the health and welfare of human beings .Some are beneficial and some are detrimental .plants are used as medicines since time immemorial .Antibacterial and antifungal properties of various plants parts like leaves, seeds, and fruits have been well documented for some of the medicinal plants for the past two decades. Antibiotic principles are the distributed widely among angiosperm plants .A variety of compounds is accumulated in plants parts accounting for their constitutive antimicrobial activities **(Vlietinck and Lindsay ,1995)**

2. MATERIALS AND METHODS

2.1 LEAF EXTRACTION

The leaves of ***Morinda citrifolia ,Euphorbia hirta, Gymnema sylvestre, Pterocarpus santalinus*** were collected from in and around areas of Chennai, Tamil nadu, India. The collected leaves were washed with tap water and dried .After they were powder.The powder was soped with methanol .filtered the extracts, The extracts were dried and dissolved in DMSO [dimethyl sulfoxide] solution and screened for antimicrobial and antioxidant activity .

2.2 ANTIMICROBIAL ACTIVITY

The antimicrobial activities was done by using bacterial strains ***Staphylococcus aureus, Escherichia coli*** and fungal strain like ***Aspergillus niger ,Candida albicans*** .All the strains were collected from microbiology laboratory .The antimicrobial activity was determined by disc diffusion methods **(Bauer et al.,1966)** .Five different concentrationof 1000,µl,500µl,250µl,12.5µg,62.5µ, respectively were prepared .Each sterile disk was loaded 20µl of test extract and placed on incubated with respective micro organisms.The negative control 20µl DMSO and positive control streptomycin(10µg)and placed on MHA plates. Then the plates were kept for incubation at 37ºC for 24 hrs for bacteria and 48 hrs for fungi.At the of incubations zones around the discs were measured .The study was performed in triplicate .

2.3 DETERMINATION OF ANTIOXIDANT ACTIVITY

The evaluation of radical scavenging activity (antioxidant activity) was conducted by the method of **(Hatano 1988 and Bhuiyan 2009)** with some modifications.The following concentrations of leaf extracts were prepared 100,200,300,400 and 500µg/ml, respectively.A stock solution of the sample(100µg/ml) was diluted up to five concentratios. Each concentration was tested in triplicate samples.The portion of sample solution was mixed with 3.0ml of ethanol.0.3ml of 0.5mM (DPPH, in 95% distilled ethanol)and allowed to stand at room temperature for 30min under light protection.The absorbance was measured at 517 nm spectrophotometrically.

2.4.1 DETERMINATION OF ANTICANCER ACTIVITY

The cancer activity of samples on VERO & MCF7 cellswere determined by the MTT assay **(Mosmann et al.,1983).** Cells (1×10^5/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO_2 incubator for 72 hours. Then, added various concentrations of the samples in 0.1% DMSO for 24 hrs at 5 % CO_2 incubator. View the images under Inverted microscope 40X and take the photos. After removal of the sample solution and 20µl/well MTT reagent was added. Viable cells were determined by the absorbance at 540nm. 50% inhibition of cell viability (IC50) value was determined graphically. The effect of the samples on the proliferation of VERO& MCF7 cells was expressed as the % cell viability, using the following

3.RESULTS AND DISSCUSION

3.1ANTIMICROBIAL ACTIVITY

Antimicrobial activity of the four samples were determined by antibiotic disc method on Muller Hinton agar(MHA) medium .The four different plants were tested for antibacterial and antifungal activities on microbial strains were presented in Table 1and 2.

The ***Morinda citrifolia, Euphorbia hirta , Gymnema sylvestre , Pterocarpus santalinus*** were found to be the highest zone were observed in methonalic extract in 10mg/ml concentration was against ***Staphylococcus aureus ,Escherichia coli*** like fungi ***Aspergillus niger, Candida albicans***

3.2 ANTIOXIDANT RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT

Antioxidant properties and other bioactivities of secondary metabolites of plants are of great interest in many fields such as pharmacology and food industries.it is growing tendency that natural antioxidant compounds are being used to replace synthetic antioxidants due to their side effects. Free redical mediated oxidavtive stress is believed to be the primary cause of many diseases and disoreders. Hence ,therapy using free-redical scavengers(antioxidant)has a potential to prevent delay or ameliorate many of these disorders. In the present study ,the methanolic extract of 100mg/ml concentraction showed a higher radical scavenging activity (73.3%) corresponding increase in absorbance is noted in extracts as well as standard when the concentractions of extract and standard were increased Table 3and 4.

3.3ANTICANCER ACTIVITY

Calculation

The selected plant extracts were tested for their cytotoxicity against normal vero cells (African green monkey kidney fibroblast) in 96 well microplates. Vero cell suspension (190 µ) containing 1.0×105cells/ml and 10µ of tested sample were added into each well.After incubation in 5% CO_2 at 37ºC for 72h ,the cytotoxicity was determined by colorimetricmethod as described by **(Skehan et al 1990).** The cell line works shown that Table 5.

Eq 1. % cell viability = A540 of treated cells/ A540 of control cells ×100%

THE COMPARATIVE STUDY ON ANTIMICROBIAL ACTIVITY

3.4 ANTIBACTERIAL ACTIVITY OF ISOLATED MICROORGANISMS

Table 1. Antibacterial activity of isolated bacteria

Bacteria	Plant extract	Zone of incubation in mm						
		62.5 µg	120 µg	250 µg	500 µg	1000 µg	Control	Antibiotic
Staphylococcus aureus	*Euphorbia hirta*	6		12	13	14	-	16
	Gymnema sylvestre	6	6	6	6	6	-	16
	Morinda citrifolia	-	6	6	6	6	-	16
	Pterocarpus santalinus	7	7	8	9	10	-	16
Escherichia coli	*Euphorbia hirta*	9	10	10	12	14	-	20
	Gymnema sylvestre	-	9	9	10	6	-	20
	Morinda citrifolia	-	-	9	9	6	-	20
	Pterocarpus santalinus	6	6	9	9	10	-	20

Fig 1 ***Staphylococcus aureus***

Table 2.Antifungal activity of isolated fungi

Fungi	Plant extract	Zone of incubation in mm						
		62.5 µg	125 µg	250 µg	500 µg	1000 µg	Control	Antiboitic
Aspergillus niger	*Euphorbia hirta*	7	7	11	13	15	-	-
	Gymnema sylvestre	-	-	-	7	10	-	-
	Morinda citrifolia	-	-	-	9	13	-	-
	Pterocarpus santalinus	-	-	9	10	12	-	-
Candida albicans	*Euphorbia hirta*	-	-	-	-	-	-	10
	Gymnema sylvestre	6	6	6	6	7	-	10
	Morinda citrifolia	6	6	6	6	6	-	10
	Pterocarpus santalinus	-	-	-	-	-	-	10

Fig 2 ***Aspergillus niger***

4.3 DPPH. RADICAL SCAVENGING ACTIVITY OF METHANOLIC EXTRACT:

Miller *et al.*, 1993 Brand-Williams *et al.*, 1995; Fogliano *et al.*, 1999.Free radicals are involved in the propagation of lipid (LH) oxidation, and many radical species of different reactivity are formed (e.g. _OH, O2__, LOO_, LO_, L_, etc.). Relatively stable radicals (DPPH.) are often preferred in the assessment of radical scavenging

Eq 2. % Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100

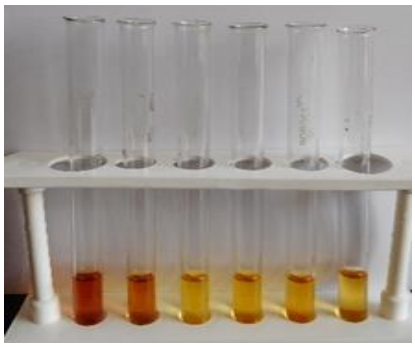
Table. 3 Standard –BHT-1mg/ml

Blank -0.59

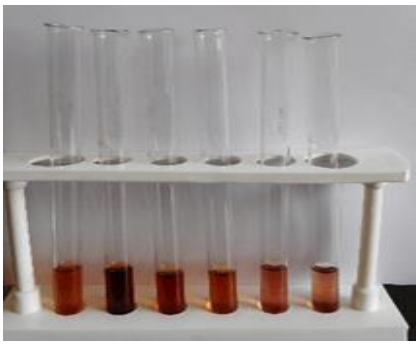
BHT Concentration	100 µg	200 µg	300 µg	400 µg	500 µg
O.D	0.36	0.27	0.17	0.15	0.11
% inhibition	38.9	54.2	71.1	74.5	99.8

Blank -0.59

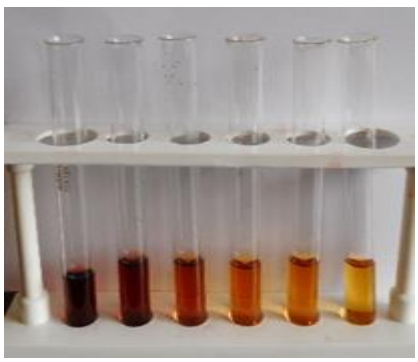
Concentration(µg)	100	200	300	400	500
Euphorbia hirta	0.28	0.26	0.24	0.23	0.20
% inhibition	52.5	55.9	59.3	61.0	66.1
Gymnema sylvestre	0.51	0.49	0.47	0.45	0.44
% inhibition	13.5	16.9	20.3	23.7	25.4
Morinda citrifolia	0.50	0.48	0.47	0.45	0.43
% inhibition	15.2	18.6	20.3	23.7	27.1
Pterocarpus santalinus	0.43	0.37	0.32	0.28	0.25
% inhibition	27.1	37.2	45.7	52.5	57.6



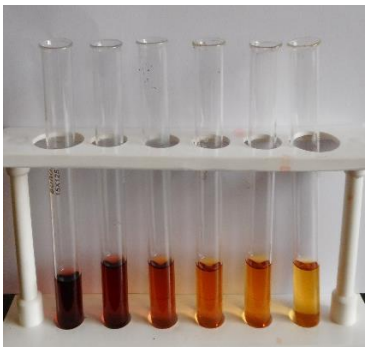
E.hirta



G.sylvestre



M.citrifolia



P.santalinus

Fig 3.DPPH assay

4.4 ANTICANCER ACTIVITY

The selective plant extracts were tested for their cytotoxicity against .MCF-7(Human breast adenocarcinoma,)by colorimetric cytotoxicity assay that measured cell growth from cellular protein content and NCI-H187(Human small cell lung carcinoma ,ATCC CRL-5804) by the produce as describe by plumb et al.(1989).Ellipticine and doxorubicin were used as positive controls and DMSO was used as a negative control .Cytotoxicity was expressed as IC50 value which is the concentration of extract needed to inhibit growth by 50% **(Skehan et al,1990).**

4.4.1 **VERO cell**

S.No	Concentration µg/ml	Absorbance 540nm	% cell Viability
1	100	0.13	10.8
2	50	0.35	29.1
3	25	0.56	46.6
4	12.5	0.97	80.8
5	6.25	1.13	94.1
6	3.12	1.18	98.3
7	Control Cells	1.20	100

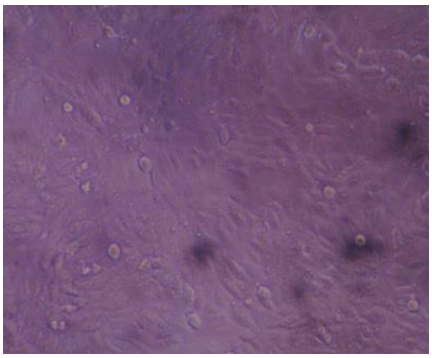


Fig 4 Vero cells

CONCLUSION

In the study,these necessity to introduce new ,biologically safe and active drugs.Naturally the plants posess biologically effective antimicrobial and antioxidant agents. The methonolic leaf extract of *Morinda citrifolia,Euphorbia hirta,Gymnema sylvestre, Pterocarpus santalinus* showed good activity against the bacterial strains of namely *Staphylococcus aureus, Escherchia coli*,and fungal strains of namely *Aspergillus niger, Candida albicans* and antioxidant studies namely DPPH. It indicates that the plant leaf contains phytochemical(medicinal) compounds for curing the different human disease and further investigation should be needed to screen the phytochemicals which are useful for pharmacological studies.

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