Journal of Chemical and Pharmaceutical Sciences ISSN: 0974-2115

ANTIOXIDANT ACTIVITY AND WOUND HEALING POTENTIAL OF SELECTED MEDICINAL PLANTS

Balachandar R[1], Saran Prakash L[1], Ashok Kumar K[1], A.Ragavi[1], *Gurumoorthy P[1]
[1] Department of Biotechnology, Arulmigu meenakshi amman college of Engineering
Vadamavandal, Thiruvanamalai district
***Corresponding Author** - Email: biogp77@gmail.com.

ABSTRACT

Plant derived drugs have been a part of the human in the healthcare for thousands of years. Throughout the world a huge percentage of population depends upon the use of plant based medicine because of their easy availability and also due to the lacks of better health care alternatives. Herbal medicines have been the basis of treatment for various diseases in traditional methods practiced such as Ayurveda, Unani and Siddha. Plant-based medicines are effective and safe without any side effects. A large number of plant extracts decoctions or pastes are equally used in India for treatment of cuts, wounds, and burns. Wounds are the result of injuries to the skin that disrupt the soft tissue. Plants and their extracts have immense potential in treatment of wounds. Several medicinal plants have shown to play significant role in healing of wounds over the years. In this study the leaves of plants *Cathranthus rosesus, Sesamun indicum Terminalia chebula* were assessed for their antioxidant and wound healing activity. The present study showed all the selected plants leaf extracts exhibited antioxidant activity which was assayed using DPPH free radical scavenging assay. All the selected extracts were subjected to cytotoxicity assay against Vero cell lines. *Terminalia chebula* extracts showed better activity than other two extracts and was selected for the wound healing assay. The wound healing study showed leaves of *Terminalia chebula* possess wound healing property.

KEYWORDS: Antioxidant, *Terminalia chebula*, cytotoxicity, and wound healing

1. INTRODUCTION

Herbal drugs play a role in the management of various disorders; most of them speed up the natural healing process of humans.Herbal medicines have been the basis of treatment and cure for various diseases and physiological conditions in traditional. Methods practiced such as ayurveda, unani and sidhha .Medicinal components from Plants an important role in conventional as well as western medicine. Numerous medicinal plants and their formulations are used for various disorders in ethano-medical practices as well as traditional system of medicine in India. Mostly herbs Contain polyphenols which are most powerful natural antioxidant and are highly valued for their antioxidant and anti-aging, Antioxidants are noted to significantly prevent tissue damage that stimulates Wound healing process *Terminalia chebula* is a member of family *Combretaceae*, it is widely used in ayurveda and genus Terminalia is commonly known as Kadukai in tamil has the potential for not only wound healing properties but also it heals digestion and constipation related problems. *Sesamum indicum* is a member of family *Pedaliaceae*. Sesame oil obtained from the seeds of Plant is highly nutrient as it is rich source of natural oxidant such as sesamin and sesamol. *Catharanthus rosesus* plant is a key source of monoterpenoidinol alkaloid, vincristine and vinblastine which found useful in treatment of cancer. In a study of methanolic leaf extract .It possess wound healing property

In our present investigation the use of *Terminalia chebula, Sesamum indicum, Catharanthus rosesus*, for the medicinal purpose used locally in the treatment of various disease and we examined these plant extracts for their wound healing, antimicrobial and antifungal activity and Cytotoxity test were carried out on Vero cell lines and A-549 cancer cell lines, therefore the present investigation is part of the continuing program related to Phytochemical analysis

2. MATERIALS AND METHODS

2.1 Collection of Plants Sample: Fresh plant leaves of *Cathranthus rosesus, Sesamum indicum, and Terminalia chebula* from Siddha University, Chennai, India

2.2 Extraction of Samples: 25 gms of fresh leaves were taken and extracted with 250ml of ethanol (Himedia Mumbai) using soxhelet apparatus for 2 hours. The extractions were then allowed to run for 5 cycles. The extract were collected in a sterile beaker and allowed for drying. Then the extract was scrapped from the beaker and stored in sample vials.

2.3 Antibacterial activity of Plant Samples: Antibacterial activity of plant extracts was determined by disc diffusion method on bacterial isolates *Bacillus subtilis* and *Vibrio* sp using Muller Hinton agar (MHA) medium. Muller Hinton Agar (MHA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the bacterial suspension. The disc were placed in MHA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc .The plates were incubated at 37ºC for 24 hrs, in upright position and the zones of inhibition formed were measured using a ruler (Kekuda, 2013)

2.4 Antifungal activity of Plant Samples: Antifungal activity of plant extracts was determined by disc diffusion method on fingal strains *Candida albicans* and *Trichophyton* using Sabouraud Dextrose agar (SDA) medium. Sabouraud Dextrose agar (SDA) medium is poured in to the petriplate. After the medium was solidified, the inoculums were spread on the solid plates with sterile swab moistened with the fungal suspension. The disc were placed in SDA plates and add 20 µl of sample (Concentration: 1000µg, 750µg and 500 µg) were placed in the disc. The plates were incubated at 37ºC for 24 hrs. in upright position and the zones of inhibition formed were measured using a ruler (Kekuda, 2013)

2.5 Radical Scavenging Efficiency of Plant Extract: The radical scavenging nature of plant extracts were evaluated by DPPH (2, 2-diphenyl-1-picrylhydrazyl) free scavenging assay. Here, 3.7ml of different concentrations of extracts and reference standard

ascorbic acid (6.25-100 µg/ml of methanol) was mixed with 3.7ml of DPPH solution in clean and labeled tubes. The tubes were incubated for 30 minutes at room temperature at room temperatures in dark. The absorbance was measured at 517nm using UV-Spectrophotometer. The radical scavenging activity of each concentration of extracts was calculated using the formula scavenging activity (%) = [(A-B)/A]*100, where A is the absorbance of DPPH and B is the absorbance of DPPH (Kekuda, 2013)

2.6 Procedure for (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazoliumbromide) MTT assay: The Cytotoxicity of samples on VERO cells was determined by the MTT assay (Mosmann T.R, 1983). Cells (1 × 105/well) were plated in 100 µl of medium/well in 96-well plates. After 48 hours incubation the cell reaches theconfluence. Then, cells were incubated in the presence of various concentrations of the samples (*Cathranthus rosesus, Sesamum indicum, and Terminalia chebula* leaf extract) in 0.1% DMSO for 48h at 37°C. After removal of the sample solution and washing with phosphate buffered saline (pH 7.4), 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide cells(MTT) phosphate- buffered saline solution was added. After 4hrs incubation, 0.04M HCl/ isopropanol were added. Viable cells were determined by the absorbance at 570nm with reference at 655nm.Measurements were carried out in triplicate, and the IC50 was determined graphically. The absorbance were measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without sample as blanks. All experiments were performed in triplicates

3. RESULTS

3.1 Antibacterial activity of selected plants: Table 1 shows the results of antibacterial activity of selected plants against Gram positive bacteria *Bacillus subtilis* and Gram negative bacteria *Vibrio spp*, for antibacterial activity.

Table 1 Shows the Antibacterial activity of selected plants

Organism	Zone of Inhibition in (mm)								
	Cathranthus roseus (*Conc* µg/ml)			*Terminalia chebula* (*Conc* µg/ml)			*Sesamum indicum* (*Conc* µg/ml)		
	1000	**750**	**500**	**1000**	**750**	**500**	**1000**	**750**	**500**
Bacillus subtilis	10mm	7mm	5mm	8mm	5mm	4mm	9mm	6mm	5mm
Vibrio spp	15mm	4mm	8mm	10mm	6mm	4mm	10mm	4mm	5mm
DMSO	0	0	0	0	0	0	0	0	0

3.2 Antifungal activity of selected plants: Table 2 shows the results of antifungal activity against *Candida Spp* and *Trichophyton* No inhibitory activity was observed in case of DMSO.

Table 2 shows the Antifungal activity of selected plants

Organism	Zone of Inhibition in (mm)								
	Cathranthus roseus (*Conc* µg/ml)			*Terminalia chebula* (*Conc* µg/ml)			*Sesamum indicum* (*Conc* µg/ml)		
	1000	**750**	**500**	**1000**	**750**	**500**	**1000**	**750**	**500**
Candida Spp	3mm	2mm	5mm	4mm	3mm	2mm	5mm	4mm	2mm
Trichophyton	5mm	3mm	4mm	3mm	4mm	2mm	4mm	3mm	2mm
DMSO	0	0	0	0	0	0	0	0	0

3.3 Radical scavenging test Efficiency of extracts of selected plants: The results of DPPH radical scavenging activity of all extracts are shown in the Figure 1 highest radical activity was shown in *Terminalia chebula* and least radical activity was shown in *Cathranthus roseus.*

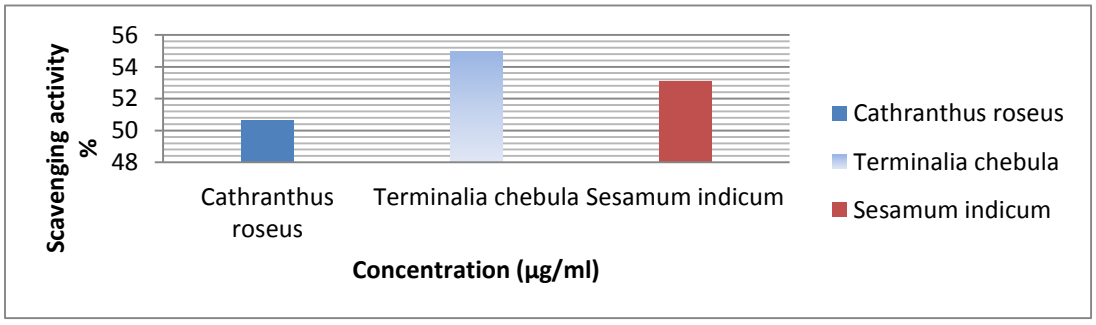
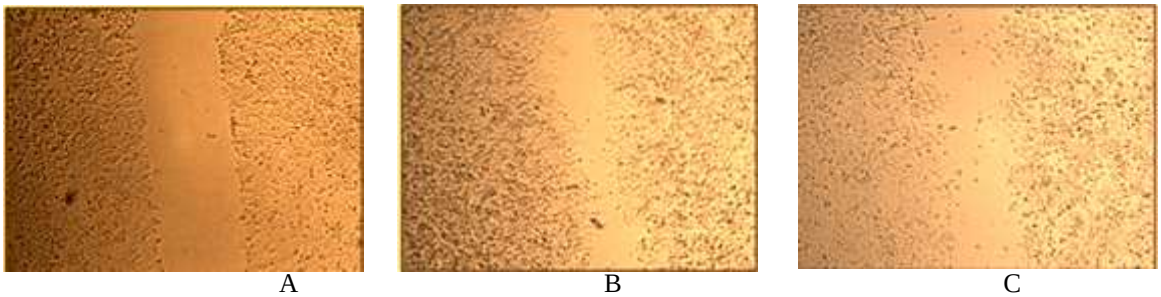


Figure 1 Shows the Radical scavenging test Efficiency of extracts of selected plants

3.4 Wound healing assay: The wound healing assay, was conducted using MTT assay on VERO cell line and the results for VERO cell line IC50 values showed highest activity in*Terminalia chebula*, *Cathranthus roseus* and *Sesamum indicum* was found to be 50.9, 49.2, 49.20 µg/ml respectively and Vero cell line IC50 values showed highest activity in *Terminalia chebula*, *Cathranthus roseus* and *Sesamum indicum*52.54, 50.8, 49.15 µg/ml respectively in Table 3, (Figure 2) picture's showed the wound healing process on VERO cell lines

Table 3 Cytotoxity effect of Sample *T.Chebula* on VERO cell line

S.No	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell viability (%)
1	1000	Neat	0.18	28.57
2	500	1:1	0.24	38.09
3	250	1:2	0.31	49.20
4	125	1:4	0.36	57.14
5	62.5	1:8	0.39	61.90
6	31.2	1:16	0.42	66.66
7	15.6	1:32	0.45	71.42
8	7.8	1:64	0.48	76.19
9	Cell control	-	0.63	100



A B C

Figure 2 Shows the Wound healing properties on VERO cell lines

A. Wound created on VERO Cell line at 0 Hrs; B - after 4 hrs; C - Wound healed by sample after 8 hrs

4. DISCUSSION

*T.chebula*is a significant medicinal plants found in Indian traditional medicine. The dried leafs were extracted with ethanol and were recovered using soxhelet apparatus and the plant extracts showed a wide variety of antibacterial activity against gram positive and gram negative bacteria strains showed diverse patterns of inhibition (Table 2), this was supported by an earlier study on an alcoholic extract that exhibited greater activity than the aqueous and hexane extracts against bacteria, with no cellular toxicity (Ahmad, 1998)and antifungal activity also showed a wide spectrum against fungal strains (Table 3). The *T.chebula* methanolic extract was evaluated using scavenging assay, reducing power and total antioxidant capacity shown in figure 1. The Cytotoxity studies on VERO cell lines using MTT assay showed broad spectrum of wound healing properties of *T.chebula*, in the MTT assay test it showed cell viability of 49.20%.Our study come in line with that of (Ramasamy, 2012) which showed the wound healing property of *T.chebula* plant on wound excised rabbits. This proved the theory that T.chebula has effective wound healing property due to the presence of high tannin content which is responsible for the wound contraction and ephithelization hence it can be inferred that the MTT studies on VERO cell lines showed greater effective wound healing process.

REFERENCES

Aebi, H. Catalase. In: Bergmeyer, H.U. (Ed.), Methods in Enzymatic Analysis, vol. 3. Academic PressInc., New York, pp.(1974) 673–686.

Aliyeva, E., Umur, S., Zafer, E. and Acigoz, G. The effect of polylactide membranes on the levels of reactive oxygen species in periodontal flaps during wound healing. Biomaterials 25:(2004)4633–4637

Baboir, B. M. Oxygen dependent microbial killing by phagocytes (first of two parts). New England J.Med. 29:(1978)629–668

Banerjee, R.D. and Sen, S. P. Antibiotic activity of Bryophytes. The Bryologist 82:(1978) 141–153

Basile, A., Giordano, S., Lopez-Saez, J.A. and Cobianch, C. Antibacterial activity of pure flavonoids isolated from mosses. Phytochem. 52: (1999)1479–1482.

Chatterjee, T.K. and Chakravorty, A. Wound healing properties of the new antibiotics (MT81) in mice.Indian Drugs 30:(1993)450–452.

Chattopadhyay, D., Arunachalam, G., Mandal, A. B., Sur, T. K., Mandal, S. C. and Bhattacharya, S. K.,.Antimicrobial and anti-inflammatory activity of folklore: *Mellotuspeltatus*leaf extract. J.Ethnopharmacology. 82:(2002)229–237.

Griendling, K. K., NADPH oxidase: role in cardiovascular biology and diseases. Circulation Res. 86:(2000)494–501

Ielpo, M. T. L., Sole, P. D., Basile, A., Moscatiella, E. L., Costaldo, R., Cobianchi, C. and Vuotto, M. L.,. Antioxidant properties of *Lunulariacruciata*(Bryophyte) extract. Immunopharmacol. Immunotoxicol.20 :(1998)555–556.

Kapil, A, Koul, I. and Suri, O. P.,.Antihepatotoxic effects of chlorogenic acid from *Anthocephaluscadamba*. Phytother. Res.9(3):(1995) 189-193.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R. I.Protein determination using Folinciocalteaureagent. J. Biol. Chem. 193: (1951)438–448.

Madsen, G. C. and Pates, A. L. Occurrence of antimicrobial substances in chlophyllose plants growingin Florida. Botanical Gazette 113:(1952)293–300.

Neuman, R. E. and Logan, M. A. The determination of hydroxyproline. J.Biol. Chem. 184:(1950).299–306.

Niranjan P. Sahua, Kazuo Koike, ZhonghuaJia, Sukdeb Banerjee, Nirup B. Mandal and Tamotsu Nikaido., Triterpene glycosides from the bark of *Anthocephaluscadamba.* J. Chem. Res.1(1): (2000). 22–23.

Okhawa, H., Ohishi, N. and Yagi, K., Assay for lipid peroxides in animal tissues by thiobarbituricacidreaction. Analytical Biochemistry 95:(1979)351-355.

Ramasamy Thangavelu, Narendhirakannan, Jesuthankaraj , Grace Nirmala, Arunagiri Caroline, Suneera Lincy, Melda Saj, Divya Durai, Evaluation of antibacterial, antioxidant and wound healing properties of seven traditional medicinal plants from India in experimental animals. Asian pacific Tropical journal of tropical biomedicine (2012) S1245 – S1253

Sidhu, G. S., Mani, H., Gaddipatti, J. P., Singh, A. K., Seth, P., Banaudha, K. K., Patnaik, G. K. andMaheshwari, R. K., Curcumin enhances wound healing in streptozotocin induced diabetic rats andgenetically diabetic mice. Wound Repair Regeneration 7: (1999). 362–374.

Slkar, I. V., Kakkar, K.K., Chakre, O. J. Glossary of Indian Medicinal Plants with Active Principles. CSIR, New Delhi, Part I, (1992). 75 p.

Sofowora, E. A., Medicinal Plants and Traditional Medicine in Africa. Wiley, Chichester,(1982). p. 256.

Thiem, B. and Grosslinka, O. Antimicrobial activity of *Rubuschamaemorus*leaves. Fitoterapia75: (2003). 93-95.

Udupa, D., Kulkarni, R. and Udupa, S. L. Effect of *Tridaxprocumbens* extracts on wound healing.Intern.J. Pharmacol. 33: (1995). 37–40.