

Abundance Of Radionuclides And Antibacterial Activity Of Medicinal Plants Against Selected Human Pathogenic Bacteria

Umesha Reddy K¹, Femila Komahal F², Kaliprasad C S^{3*}, Srilatha M.C⁴, K N Narasimha Murthy⁵, Sreedhar R⁶, Suresh C⁷, Laveena K B^{8*}

¹Department of Physics, Government First Grade College, Hoskote- 562114.

²Department of Physics, Nrupathunga University, Bangalore-560001.

³Department of Physics, BMS College of Engineering, Bangalore-560019, India

^{4,7}Department of Physics, Government First Grade College, Malleshwaram- 560003

⁵Department of Physics, Government First Grade College, Yelahanka- 560064, Bangalore.

⁶Department of Physics, Government First Grade College, Hiriya - 577598, Karnataka.

⁸Department of bioscience, Mangalore University, Mangalagangothri, Mangalore -574199

*kpkaliprasad23@gmail.com, laveenavenkatesh@gmail.com

Abstract:

In the present study few medicinal plants were collected from the Western Ghats. The activity concentration of radionuclides in soil and plant leaves was measured using gamma ray spectrometer. The antimicrobial activity was measured by Agar well diffusion assay. The result of the antibacterial study showed the presence of wide spectrum of antibacterial activities against bacterial pathogens. Calculated transport factor and risk assessment in medicinal plants, which shows good antibacterial activity even though the high radionuclides concentration.

Keywords: Antimicrobial, Medicinal plants, Radionuclides, Radium, Thorium, Transport factor

INTRODUCTION

Medicinal plants are unique type of natural product requiring special consideration due to their potential impact on human health. The pharmacological properties of the medicinal plants have been attributed to the presence of active chemical constituents which are responsible for important physiological function in living organisms (Fahad *et al.*, 2014). According to the International Food Safety Authorities Network, plants used as food commonly have ⁴⁰K, ²³²Th and ²³⁸U and their progenies (INFOSAN, 2011). It is expected that similarities would be found in plants used for medicinal purposes since plants are the primary pathway of natural radionuclides entering into the human body through the food chain. In many developing countries, pollution is the main causes for the contamination of vegetation with heavy metals, pesticides, or radioactivity. Therefore the medical properties and effectiveness of medicinal plants depend on environmental conditions, which also have a direct influence on their growth in the place of their occurrence and cultivation (Salaman, 2004). The medicinal quality of plants depends on their chemical constituents that have physiological activity in human systems (Kumar *et al.*, 2009). Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites (Cowan, 1999). Microorganisms that form a parasitic association with other organisms are classified as pathogens. Some bacteria and fungi can be pathogenic while some are not. The search for novel potent plants and plant components against pathogenic microorganisms has become increasingly important, as a result of the development of antibiotic resistance in microbes (Ahmad *et al.*, 2006). Antimicrobial investigation of plant extracts and phytochemicals is the basis for antimicrobial drug discovery (Cseke *et al.*, 2006; Innocent and Chinelo, 2018). Therefore in the present investigation selected the medicinal plants to study the presence of concentration of radionuclides and to assess the radiological risk associated with the use of selected medicinal plants viz., *Alternanthera ficoidea* L., *Erythrina variegata* L., *Mussaenda belilla* L. and *Sauropus androgynus* L. which has great significance and also to study the antibacterial activity of *A. ficoidea* and *M. bellila* against human bacterial pathogens.

MATERIALS AND METHODS:

Sample Collection and Preparation of Extraction:

The medicinal plants and associated soil samples were collected from different regions of Western Ghats following standard procedure (EML 1983). The collected medicinal plants, its common name, uses and parts used were tabulated in table 1. The leaves of these plants were washed under running tap water and rinsed well with distilled water, oven dried (40° C) and powdered. For extraction with, 25 g of powdered plant material was dissolved in enough sterilized ethanol and water to make 100ml of ethanol and aqueous extract (25% w/v). The mixture was kept undisturbed at room temperature for 24 hrs in a sterile flask covered with aluminum foil to avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25 ml extract was left in the container. Extracts thus obtained were immediately evaluated for antibacterial using agar well diffusion method (Chen *et al.*, 1987, Barreto *et al.*, 2002). The soil samples were dried in hot air oven till constant weight is obtained and samples were processed as per EML standard procedure (EML. 1983). The dried samples were sieved through 250 micron mesh and stored in PVC container for 30 days to obtain secular equilibrium between Radium-226 and its daughter products.

Table 1. Scientific name and uses of selected traditional medicinal plants

Sl.No	Scientific Name	Common Name	Medicinal uses	Part(s) used
1	<i>Erythrina variegata</i> L.	Indian coral tree	It is used especially for menstrual disorders and fissures at penis tip.	Leaves
2	<i>Sauropus androgynus</i> L.	Vitamin plant	Used as Vegetable with high antioxidant potential	Leaves
3	<i>Alternanthera ficoidea</i> L.	Joseph's coat	The plant might be useful as an antiviral agent.	Leaves
4	<i>Mussaenda belilla</i> L.	Wild mussaenda	A decoction of the leaves is used to rid the body of intestinal worms.	Leaves and sepals

Activity measurement

The activity concentration of radionuclides in soil and plants samples were measured using NaI(Tl) based gamma ray spectrometer with standard procedure. The size of detector is 5 cm × 5 cm was used along with Multichannel Analyser. The detector was shielded with lead blocks all the four sides to reduce backgrounds counts due to terrestrial gamma ray radiations. The spectrometer was calibrated using RG-U, RG-Th and RG-K, as standard Uranium, Thorium and Potassium sources procured from International Atomic Energy Agency, Vienna. The full-width half maximum (FWHM) was 60.78 KeV with resolution 8.46%, for the 137Cs (661 KeV) peak. The samples were analyzed with an acquisition time of 80,000 s, using GSPEC software to obtain the gamma-ray spectrum with good statistics. The activity concentration of ²²⁶Ra was estimated from 1764 KeV (15.9%) gamma peaks of ²¹⁴Bi. ²³²Th activity was estimated using 2614 KeV (35.8%) gamma transition of ²⁰⁸Tl. ⁴⁰K radionuclide was estimated using 1460.8 KeV (10.7%) gamma peak from ⁴⁰K itself to determine the concentration of ⁴⁰K in different samples (Alnour *et al.*, 2012; Harb, Abbady, El-Kamel and El-Mageed, 2012; Shams, Uosif, and Abd, 2011; Venunathan *et al.*, 2016).

Antibacterial Activity

Agar well diffusion assay

The leaf extracts were first subjected to standard analysis for the presence of phytoconstituents as described by Harborne, 1988. The antimicrobial activity was measured by Agar well diffusion assay (Perez *et al.*, 1990). The *A. ficoidea* and *M. belilla* plant extracts were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Petri plates containing 15 ml Nutrient agar medium were seeded with the bacterial strains. Each labelled medium plate was uniformly inoculated with a test organism by using a sterile cotton swab rolled in the suspension to streak the plate surface in a form that lawn growth can be observed. Wells were punched and 25, 50, 75 and 100µl of the methanolic plant extracts were added. The plates were then incubated at 37 °C for 24 hours. Chloramphenicol (0.05%) was used as positive control. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. The diameter of zone of inhibition was measured in millimetres (Shakouie *et al.*, 2012).

RESULT AND DISCUSSION

The activity concentration of radionuclides in soil and plant leaves was measured using gamma ray spectrometer as shown in table 2 and 3. The activity concentration of ^{40}K in medicinal plants ranges from 34.1 Bq kg⁻¹ to 117.1 Bq kg⁻¹ and ^{226}Ra ranges from 3 Bq kg⁻¹ to 8.3 Bq kg⁻¹ for different plants. The activity concentration of ^{232}Th ranges from 9.8 Bq kg⁻¹ to 16.4 Bq kg⁻¹. The variation in activity concentration was observed from plant to plant and soil to soil. The variation may be due to the presence of minerals in the soils and absorption rate of plants. Hence, the transfer factor (TF) was calculated to know the accumulation of radionuclides by plant leaves and other parts of the plants and also to know the rate of absorption by the plant. The transfer factor has been calculated for radionuclides from soil to plant as shown in table 4. The transfer factor value ranges from 0.08 to 1.32 for different radionuclides and plants. Results predict that transformation from soil to plant leafs, potassium has highest average TF factor. This is because potassium is one of the important element to plant fertility, even though potassium is a radioactive element. Extracts from leaf samples are important in treatment of various illnesses. So the study showed that these leaves have low TF of the radionuclides.

Table 2: Activity concentration of Radionuclides in Medicinal Plants

Sl.No	Medicinal plants	Radionuclides Activity (Bq kg ⁻¹)		
		^{40}K	^{226}Ra	^{232}Th
1	<i>E. varigata</i>	43.3	3.00	9.8
2	<i>S. androgynus</i>	34.1	4.8	8.8
3	<i>A. ficoidea</i>	117.1	5.8	16.4
4	<i>M. belilla</i> (leaves)	61.8	4.9	6.4
5	<i>M. belilla</i> (sepals)	34.3	8.3	11.2

Table 3: Radionuclides Activity concentration of Soil Sites

Sl.No	Soil sites	Radionuclides Activity (Bq kg ⁻¹)		
		^{40}K	^{226}Ra	^{232}Th
1	Mangalore University Campus	88.5	12.9	23.0
2	Western Ghats of Karnataka	49.8	7.4	16.8

Table 4. Transfer factor from soil to plant

Sl. No	Medicinal plants	Transfer factor ratio soil to plant		
		⁴⁰ K	²²⁶ Ra	²³² Th
1	<i>E. varigata</i>	0.87	0.40	0.58
2	<i>S. androgynus</i>	0.69	0.64	0.52
3	<i>A. ficoidea</i>	1.32	0.45	0.71
4	<i>M. bellila</i> (leaves)	0.89	0.17	0.08
5	<i>M. bellila</i> (sepals)	0.69	1.12	0.66

Primary phytochemical screening revealed the presence of major groups that includes carbohydrates, proteins, carotenoids, terpenoids, flavonoids, phenolic compounds, alkaloids, tannins, saponins, pigments and minerals that have antimicrobial and antioxidant activity. The result of the antibacterial study showed the presence of wide spectrum of antibacterial activities against all the above bacterial pathogens studied shown in **table 5**. In *A. ficoidea* and *M. bellila* leaves, the maximum zone of inhibition was observed in ethanolic extract of leaf compared to aqueous extract for each bacterium. *M. bellila* sepal showed maximum zone of inhibition in aqueous extract compared to ethanolic extract. It was compared against radionuclides transport factor ratio in leaf and sepal against soil. In this study the average activity concentration obtained for ⁴⁰K is less which did not show any effect on antibacterial activity of plants. The mean activity of radionuclides in plant shows no risk to the consumption of plant.

Table 5: Antibacterial Activity of *A. ficoidea* and *M. bellila* against selected pathogenic bacteria (Diameter of Zone of inhibition in mm)

Plant Species	Leaves Extracts	-ve Contr ol	<i>Staphylococcus aureus</i>				<i>Bacillus subtilis</i>			
			25µl	30µl	35µl	40µl	25µl	30µl	35µl	40µl
<i>Alternanthera ficoidea</i>	Aqueous	0	20mm	20mm	18mm	20mm	21mm	22mm	19mm	17mm
	Ethanol ic	0	16mm	14mm	17mm	21mm	17mm	16mm	16mm	20mm
<i>Mussaenda bellila</i> (leaf)	Aqueous	0	-	-	-	-	5mm	6mm	9mm	9mm
	Ethanol ic	0	16mm	19mm	16mm	20mm	13mm	13mm	5mm	5mm
<i>Mussaenda bellila</i> (sepal)	Aqueous	0	13mm	10mm	10mm	12mm	14mm	13mm	12mm	14mm
	Ethanol ic	0	12mm	11mm	9mm	9mm	10mm	15mm	13mm	6mm
			<i>Proteus vulgaris</i>				<i>Escherichia coli</i>			
<i>Alternanthera ficoidea</i>	Aqueous	0	16mm	19mm	17mm	18mm	18mm	18mm	19mm	20mm
	Ethanol ic	0	12mm	15mm	12mm	15mm	10mm	15mm	15mm	19mm
<i>Mussaenda bellila</i> (leaf)	Aqueous	0	7mm	7mm	9mm	12mm	11mm	14mm	12mm	12mm
	Ethanol ic	0	14mm	10mm	20mm	16mm	14mm	12mm	13mm	15mm
<i>Mussaenda bellila</i> (sepal))	Aqueous	0	5mm	10mm	10mm	10mm	15mm	18mm	18mm	18mm
	Ethanol ic	0	5mm	10mm	10mm	10mm	-	-	-	-

CONTROL Chloramphenicol (0.05%)	Aqueous Ethanol ic	0 0								
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The study of medicinal plants has become the focus of research ever more extensive in all over the world, due to the diversity and potential that plants have as source of medicinal products. The presence of radionuclides in plants constitutes the pathway for their migration to the human. A medicinal plant containing high concentrations of these radionuclides can cause health problems, since medicinal plants are commonly used for long periods of oral treatment. An attempt was made to analyse the accumulation of radionuclides and antibacterial activity in a selected medicinal plants against human bacterial pathogens. The result of the present study showed the presence of wide spectrum of antibacterial activities against all the above bacterial pathogens studied. In *A. ficoidea* and *M. bellila* leaf the maximum zone of inhibition of observed in ethanolic extract compared to aqueous extract for each bacterium. *M. bellila* sepal showed maximum zone of inhibition was observed in aqueous extract compared to ethanolic extract and it was compared against radionuclides transport factor ratio in leaf and sepal against soil. The average activity concentration obtained for ^{40}K obtained in this study is less which did not show any effect on antibacterial activity of plants. The mean activity of radionuclides in plant shows no risk to the consumption of plant. The present study showed that the selected medicinal plant is potentially good sources against selected human bacterial pathogens. The activity concentration of radionulcides and antibacterial activity has been correlated to find the any relation between them and as shown in table 6. The bold letter indicates the positive correlation between the parameters. The ^{40}K shows good correlation with antibacterial activity in both aqueous and ethanolic solution. But the ^{226}Ra showing negative correlation with respect to all antibacterial activity, in ethanolic solution the correlation is highly negative.

Table 6. Correlation coefficients for radionulcides activity and antibacterial activity

TABLE OF CORRELATION COEFFICIENTS FOR DIAGONALIZATION OF $\mathbf{H}^{(1)}$ AND $\mathbf{H}^{(2)}$ IN $\mathbf{H}^{(0)}$												
		A			B		C		D			
		^{40}K	^{226}Ra	^{232}Th	a	b	a	b	a	b	a	b
	^{40}K	1.000										
	^{226}Ra	-	1.000									
	^{232}T											
A	h	0.673	0.233	1.000								
	a	0.517	0.417	0.981	1.000							
B	b	0.755	0.967	0.023	0.171	1.000						
	a	0.600	0.324	0.995	0.995	0.072	1.000					
C	b	0.994	-	0.588	0.421	0.822	0.509	1.00				
	a	0.987	-	0.423	0.783	0.647	0.640	0.720	0.96	1.00		
D	b	0.599	0.999	-	-	-	-	0.68	0.46	1.00		
	a	0.996	-	0.486	0.736	0.592	0.693	0.669	0.98	0.99	0.52	1.00
	b	0.544	1.000	-	-	-	-	0.63	0.40	0.99	0.46	1.00
								2	2	8	6	0

A- *Staphylococcus aureus*, B- *Bacillus subtilis*, C- *Proteus vulgaris*, D- *Escherichia coli*, a- Aqueous
b- Ethanolic

CONCLUSION

In the present, the risk factor due to radionuclides in the selected plants was measured. The activity concentration in the selected plants show below the world average, but the transport factor very high in *A. ficoidea* compared to the other parts of plants. The statistical analysis showed that the selected medicinal plants *A. ficoidea* L. and *M. bellila* L. are potentially good sources against selected human bacterial pathogens. Hence, the medicinal plants samples are considered safe in terms of the radiological hazard.

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