"Anti-microbial activity of roots of *Rauwolfia serpentina* (Apocynaceae) collected from different geographical regions of India"

[1,2]*Amrinder Kaur, [3]R.K Pareek

[1]*PhD Scholar, Department of Ayurveda, Desh Bhagat University, Mandi Gobindgarh, Fatehgarh Sahib, Punjab; [2]Asst. Professor, Dept. of Ayurveda, School of Pharmaceutical Sciences, Lovely Professional University, Punjab

[3]Ex-Professor and HOD (Medicine Department), SK Government Ayurvedic Collage, Kurukshetra, Haryana.

Abstract: Antimicrobials are the natural killers of micro-organisms that invade them. The main benefits of these agents are their ability to cause selective toxicity on the target without hampering the natural equilibrium. *Rauwolfia serpentina* (Sarpagandha) is a glabrous perennial undershrub. It is given in many disorders like worm infestation, insomnia, epilepsy, fever, obesity, mental ailments, influence of evil spirit etc. It is reported that the plant also has antimicrobial activity. This article deals with the effect of geographical variation on the anti-microbial property of plant. The roots are collected from different zones of India. The water as well as ethanol extracts were studied against the pathogen *Escherichia coli* in Mueller-Hinton agar culture media. The results calculated from the inhibition zone revealed that the aqueous extract of five samples of roots of *Rauwolfia serpentina* shows the highest zone inhibition against the *Escherichia coli*. The highest zone inhibition is in the sample collected from North region in both ethanolic and aqueous extracts.

Key words: Sarpagandha, *Rauwolfia serpentina*, Anti-microbial, *Escherichia coli*

Introduction:

Traditional medicinal practices employ the use of herbal plants since ancient times. According to the World Health Organization (WHO), approximately 80 percent of the population worldwide cover their primary health needs by utilizing the herbal medicines in day to day life. [1] Out of the wide variety of flora, around 21,000 plant species have demonstrated medicinal properties. Treatment involving medicinal plants is considered healthy as the adverse effects are zero to minimal.[2] They are a rich source of anti-microbial properties but a systematic investigation to accelerate their mortality and morbidity conditions is of utmost concern.[3] Geographical differences influence the percentage of active constituents that can be collected from a herbal plant during harvesting or processing conditions.

Rauwolfia serpentina commonly known as Sarpagandha is a member of the Apocynaceae family. Its first reference is found in the pre-vedic period. There is much folklore for this plant. In Bihar it is renowned as "pagal ki jaddi-buti" because of its curative effect in case of insanity.[4] Mongooses chew its leaves before fighting with cobra to strengthen its body. Sarpagandha is found in the tropical Himalyas and at moderate altitudes in North Bihar, Bengal, Sikkim, Patna, Assam, Bhagalpur, Konkan, Burma, Srilanka,

Andaman, Pegu, Tenasserim and Deccan Peninsula along the Ghats to Ceylon and Travancore, Malay Penisula and Java.[5]

It is a glabrous perennial undershrub. Leaves are in whorls of 3-4, rarely opposite, elliptic-lanceolate or obovate acute or acuminate. Light to dark green in color and soft to touch. Flowers are white, pink, red or bluish white in color and around 3cm. in size. These are arranged in bunches on the branch. Fruits are drupes, pea sized, purple black. When ripe seeds are ovoid in shape. Roots pieces are thick, curved and stout, rarely branched, sub cylindrical in shape. On breaking it is circular with centripetal lines and externally it is grayish yellow to brown in color.[6]

Major constituents are: resrepine, rescinnamine, serpentine, ajmaline, ajmalicin, ajmalicidine, rauhimbine, indobinine, reserpiline, sarpagine, serpentine, serpentinine, yohimbine, ajmalimine, ajmaline, rauwolfinine (perakenine), sandwicolidine, serpinin.[7]

Sarpgandha is given in many disorders like bharama (disorientation), krimiroga (worm infestation), anidra (insomnia), apasmar (epilepsy), vrana (injury), javara (fever), medoroga (obesity), unmand (insanity), manasaroga (mental ailments), vrana (injury), sula (pain), bhutavadha (influence of evil spirit).[8] Rauwolfia serpentina has reported anti-bacterial activity against Bacillus subtilis, Staphylococcus aureus, and Escherichia coli by disk diffusion method.

To gain maximum insight into the anti-microbial property, the plants were collected from different geographical locations of India as shown in Table 1.

Table 1: Collection of *Rauwolfia serpentina* from different geographical zones

S. No.	Sample No.	Zones
1	S1	North: Mandi (Himachal Pradesh)
2	S2	South: Banglore (Karnataka)
3	S3	East: Kolkata (West Bengal)
4	S4	West: Kota (Rajasthan)
5	S5	Random

Material and Methods:

Plant Collection and Authentication:

The collection of samples is done from four zones in India: Mandi (Himachal Pradesh), Banglore (Karnataka), Kolkata (West Bengal) and Kota (Rajasthan). One sample was collected randomly. The collected crude drugs were validated by the Department of Botanical and Environment Sciences, Guru Nanak Dev University, Amritsar. The collected samples were thoroughly rinsed off under running tap water to remove impurities and then dried in dark for removal of moisture content. Finally, it was powdered using a mixture grinder.

Preparation of extract:

250 ml of ethanol was used for the extraction of air-dried drugs with the help of Soxhlet apparatus. 250 ml of water was also used to prepare extracts. These extracts were collected in sterile bottles.

Antimicrobial Assay:

The anti-microbial behavior of various Sarpagandha samples were investigated with the help of the method of well diffusion agar. The agar sterilizing media is poured into the petri-plates and allowed to solidify. Using sterile cork borer (6 mm), wells are made into the petri-plate after solidification. After that pathogenic cultures are swabbed using sterilized cotton swabs on the respective agar plates. The ethanolic as well as water extracts are loaded into the respective wells and incubated for 24 hrs at 37^0C. After the last step of incubation, the anti-microbial activity was accessed by measuring the zone of inhibition formed around each well in terms of millimeter (mm).[9]

Result and Discussion

This study delineates the anti-microbial property of *Rauwolfia serpentina* collected from varied geographical zones of India. For this purpose, the ethanolic extract as well as the water extract of this herbal plant was obtained and its activity was analyzed using agar well diffusion method on the pathogen *Escherichia coli*. Different extracts of *Rauwolfia serpentina* demonstrated varying degree of anti-microbial activities against the pathogen. The anti-microbial activity of Rauwolfia serpentina extracts against E. coli are shown in table 1. The zone of inhibition was calculated and results revealed that the aqueous extract of five samples (S5, S6, S7, S8, S13) of roots of *Rauwolfia serpentina* shows the highest zone inhibition against the *Escherichia coli* in Mueller-Hinton agar culture media. The highest zone inhibition is in the sample collected from North region in both methanolic and aqueous extracts. Thus, the antimicrobial property of the plant can be validated from the results obtained.

Table 2: Zone inhibition in mm for alcoholic and aqueous extracts of *Rauwolfia serpentina* on E. coli

S. No.	Root extract	Control	Standard	Root extracts				
				S1	S2	S3	S4	S5
1	Ethanolic	0 mm	8 mm	14 mm	12 mm	11 mm	12 mm	10 mm
2	Aqueous			25 mm	24 mm	21 mm	22 mm	19 mm

Conclusion:

In conclusion, this study has paved a way for validating the scientific justification of the fact that *Rauwolfia serpentina* (possessing the bioactive components) demonstrated anti-microbial properties against *Escherichia coli*. Further it can be used to combat a variety of bacterial infections. However, more purifications are required in order to produce effective results which can contribute to the development of high-quality antibacterial formulations and the burden of drug resistance can be reduced in this regard. Thus, from a futuristic perspective, it is important to minimize the incompatibilities and adverse reactions which might occur in between the drugs during preparation of medicinal formulation.

References:

[1] Martins Ekor, "The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety." Frontiers in Pharmacology, 2014. 4(177): p. 1-10.

[2] Ali Karimi, Maedeh Majlesi, Mahmoud Rafieian-Kopaei, "Herbal versus synthetic drugs; beliefs and facts." Journal of Nephropharmacology, 2015. 4(1): p. 27-30.

[3] Shikha Sharma, Amrinder Kaur, Anania Arjuna, "Anti-microbial activity of Cuscuta reflexa collected in different seasons." International Journal of Pharma and Bio Sciences, 2013. 4(3): p. 1393-1396.

[4] Bapalal G Vaidya, "Nighantu Adarash." Chaukhambha Bharti Academy, Varanasi (Vol. I), p. 1-3, 864-866.

[5] KM Nadkarni, "Indian Materia Medica with Ayurvedic, Unani & Home Remedies." Vol.-I. 1st ed. Popular Prakashan Pvt. Ltd. 2007. p.1050-1053.

[6] N Tandon, M Sharma, "Quality Standards of Indian Medicinal Plants." Vol. Vlll, Indian Council of Medicinal Research; New Delhi, 2010. p. 272-280.

[7] JLN Shastry, "Dravyaguna Vijnana." Vol. ll. Chaukhamba Orientalia; Varanasi, p. 334-337.

[8] PS Vaidyaratnam, "Indian Medicinal Plants (A Compendium of 500 Plants)." Vol.-IV, Universities Press (India) Pvt. Ltd.; Hyderabad, 2010. p. 409-410.

[9] JS Negi, VK Bisht, AK Bhandari, DS Bisht, P Singh, N Singh, "Quantification of reserpine content and antibacterial activity of Rauvolfia serpentina (L.) Benth. ex Kurz." African Journal of Microbiology Research, 2014. 8(2), p. 162-166.