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Phytochemical and antimicrobial study of *Rauwolfia serpentina*

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Abstract
The ethanolic extract of powder root of *Rauwolfia serpentina* was screened in regards to the effects of the extracted phytochemicals on combating the oxidation stresses and the free radicals. Its medicinal role is well documented and recognized. The antioxidant and antibacterial properties of *Rauwolfia serpentina* are presented here. For the antibacterial activity, the action of ethanolic extract against the bacterial infections caused by 02 gram (+ve) and 03 gram (-ve) bacteria have been studied. Out of 05 bacteria tested on, only 03 bacteria (Anne, 1958, Klohs, *et al.* 1954 and Machillamy, 1963) [1, 4, 5] were found susceptible, the order being *Klebsiella pneumoniae* > *Staphylococcus (local)* > *Bacillus subtilis*. The bacterial infections caused by the bacterias appear to be treatable using ethanolic extract of *Rauwolfia serpentina*. In the study of antioxidant activity the tested concentration was raised from 50-5000(μg). The data showed the continuous increase in the FRAP value till 141.0 μm after which the FRAP value assumed nearly the constant value (180 μm), showing leveling effects.

Keywords: Black grape seeds, antioxidant activity, antimicrobial activity, FRAP method

Introduction
It has been several years ago that the drugs of vegetable origin are used in the prevention, diagnosis, treatment or cure of the diseases in man & animals. Plant derived medicines have been the first line of defence in maintaining health and combating diseases. In this current age by taking the knowledge from the past the therapies have been modified by means of advanced scientific knowledge and technology. Chemical principles and active ingredients have been isolated for the development of new drugs from medicinal plants which can then be formulated in various dosage forms. This has been done in order to reduce the unwanted material that we took along with active centre. It also provides specificity. Detailed research on the chemistry and pharmacology of drugs of plant origin has been done and is still going on.

One of the famous tranquilizer and antipsychotic herb of India for treatment of paranoia and schizophrenia as well as substance that control hypertension is *Rauwolfia serpentina* usually called Sarapgandha or 'snake root' has been pronounced as a "Wonder drug of India "in 1949 when the British heart journal reported the plant to be clinically effective in treating high blood pressure. *Rauwolfia serpentina* (L). Benth. Ex Kurz is a plant of family Apocynaceae. It is known by a variety of classical names like Sarpagandha, Chotachand, Chandrabhaga, Harkaichanda, Nai etc.

It is found distributed in India, Pakistan, Srilanka, Burma and Thailand In India, it is widely distributed in sub-Himalayan tract from Punjab to Nepal, Sikkim and Bhutan. *Rauwolfia serpentina* is an evergreen plant, perennial, glabrous erect under shrub grows up to a height of 60 cm, usually 30 to 40 cm. Roots are tuberous, with pale brown cork, zigzag with irregular nodes, 30 cm. Long. Leaves are in the whorls of three, elliptic to lanceolateor obovate, bright green above, pale green below, tip acute or acuminate, base tapering and slender and petioles long. Flowers bisexual, white often, have violet coloured tinge. They occur in many flowered irregular cymose cymes. Peduncles are long but pedicels stout. The most important plant parts to be used are roots and leaves. Roots are bitter in taste with external colour greyish yellow to brown and internal colour is whitish grey, surface is slightly wrinkled and rough with coarse longitudinal markings.

The Sarpagandha is constituted by a large number of alkaloids occurring in a range of 1.7% to 3.0 % in roots. Root bark is rich in alkaloidal content has more than 90% of the total alkaloids. The percentage of alkaloids depends upon geographical place, season of collection and plant age. Over 30 alkaloids have been isolated. The major are reserpine and ajmaline. The other alkaloids are ajmalicine, isoajmaline, ajmalinine, chandrine, rawolscine, rawolfinine, renoxidine, rescinamine, reserpilline, reserpinine, sarpagine, serpentine, serpentinine, tetraphyllicine are the minor alkaloids found in the samples of India. In regards to the therapeutic uses *Rauwolfia serpentina* has been mentioned in 'Sushruta samhita' as one that slows heart rate and induces sleep, suppresses libido (kamvaasadinee), beneficial in colic pain, fever and parasitic infections. Bhavpraksha said that this plant counter venom of snakes, scorpion etc. and heals wounds. But Bhasker and Caius have rendered it useless in the treatment of snake bite and scorpion sting. In folk lore, roots of this plant have been greatly valued for the treatment of insanity, epilepsy and related disorders. In some part of India it is called 'paagal ki booti' (plant for insane). Ayurvedic physicians also consider the plant as diuretic, laxative and an effective remedy for intestinal disorders.

In the last decades its medicinal properties has been accepted by the allopathic system. Its various pharmacological activities include anticholinergic, hypotensive (Klch and Fazekas, 1956 and Klohs, *et al.* 1954) [2, 4], anticontractile, sedative, relaxant, hyperthermic, antidiuretic, sympathomimetic, hypnotic, vasodialater, antiemetic, anti-fibrillary activity (Dhawan, *et al.* 1955) [3], tranquilizing

agent (Anne, 1958 and Macphillamy, 1963) [1,5], anti arrhythmic, antifungal nematocidal. Taking a lead from Ayurveda in India prolonged clinical trials of *Rauwolfia serpentina* root powder has been done and it is concluded that it has significant tranquilizing and antihypertensive effect (Chakravarti and Mukherjee, 1952, Essellier, *et al.* 1955, Bohner and Theobald, 1955, Jacques *et al.* 1959, Raynaud and Deshougus, 1955, Sukhdev, 1999 and Saland, 1956) [6-12]. As a result of a large number of therapeutic usefulness there is a great demand for roots of *Rauwolfia serpentina* and a large part of produce is exported. Thus it acts as a source of income to India. Hence there is a need to increase its production and conservation from economic and health point of view.

Material and Methods

Chemical and equipments

All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from sigma, Merck etc. water was used for the complete study. All the glassware and equipments used for the handling of bacterial cultures and plant extracts were sterilized prior to use. Sterilization procedure was performed by autoclaving at 121°C for 15 minutes.

Plant material

Root of *Rauwolfia serpentina* was used in this investigation. The dried roots were collected from the local market of Dehradun. The roots were then powdered by using mortar and pestle. The powdered roots were then preserved in a tight container for experiment.

Test organisms

The table given below (table -1) list the range of bacterial organisms used throughout the study. These organisms were used for the investigation of antibacterial activity of plant extracts. The bacteria were propagated using the conditions described in table-1, according to the recommendations of the supplier.

Nutrient media

All solid and liquid culture media were sterilized after preparation according to the method described below. Nutrient media were prepared to the manufacture specification and are listed in the table 2 given below-

Organic extraction

250 gm. of powdered dried roots of *Rauwolfia serpentina* were taken in a round bottom flask and 1 litre of ethanol was added. Refluxing was carried out for 48 hours. Evaporation of solvent was done upto pourable condition. The contents were then poured into a bowl, further solvent was evaporated and the extract was calculated per gram of dried material. The contents were dark brown in colour.

Antibacterial assay

The Agar well diffusion method was used to evaluate grown in the antibacterial activity and incubated at 37°C for 24 hrs. After incubation period is finished the absorbance of the culture was adjusted to 0.5 according to McFarland turbidity standard with sterile nutrient broth. The 0.02 ml. of the culture was seeded on the sterile petri plates containing sterile Muller Hinton Agar media. The well was bored with 9 mm. Borer in seeded Agar. Then the 100 (µl) of the plant extract was added in each well. Plates were then incubated at 37°C for 24 hrs. After incubation period was finished the zone of inhibition (mm.) was measured and recorded against *Bacillus subtilis* (gram + ve), Staphylococcus (local) (gram +ve), *Pseudomonas aeruginosa* (gram – ve), *Klebsiella pneumonia* (gram – ve) and *Salmonella typhimurium* (gram – ve) bacterias.

Antioxidant Activity

The antioxidant activity was measured by FRAP means the ferric reducing ability of plasma or plants.

Reagents

1. Acetate buffer: 300 m Mol / 1 pH 3.6 (3.1 g sodium acetate x 3 H2O and 16 ml acetic acid in 1000 ml. Buffer solution.
2. 10 m Mol / 1, 2, 4, 6- tripyridyl- S – triazine (TPTZ) in 40 m Mol/ 1 HCl.
3. 20 mMol / $FeCl_3.6\ H_2O$ in distilled water.

FRAP Working Solution

25 ml acetate buffer, 2.5 ml TPTZ solution and 2.5 ml. $FeCl_3.6\ H_2O$ solution. The working solution must be always freshly prepared. Aqueous solution of known $FeSO_4.7\ H_2O$ + Distilled water was used for calibration.

Assay

Blank: FRAP Reagent Monitoring up to 15 sec. at 593 nm., 1cm. Light path and 37°C, Fe (II) standard solution tested in parallel calculation using the calibration curve.

Results and Discussion

Rauwolfia serpentina – an ayurvedic drug, is having used in India for a longer time. The plant has said to constitute a large number of alkaloids. Over 30 alkaloids have been isolated. The major are Reserpine and Ajmaline. The other alkaloids are ajmalicine, isoajmaline, ajmalinine, chandrine, rawolscine, rawolfinine, renoxidine, rescinamine, reserpilline, reserpinine, sarpagine, serpentine, serpentinine, tetraphyllicine, raucaffricine. Besides, it contains steroids (17)β-methyl-5-androsten-3-β-ol, β-sitosterol and it's dehydro derivative. Also reported phytochemicals include 1,2-di- hydrouomilenine reductase, serpoterpene, ajmalicine, yohambaoid, monoterpenoid, indole alkaloid etc. Its medicinal role is well documented and recognized. The antioxidant and antibacterial properties of *Rauwolfia serpentina* are presented here.

The ethanolic extract of powder root of *Rauwolfia serpentina* was screened in regards to the effects of the extracted phytochemicals on combating the oxidation stresses and the free radicals.

Antibacterial Activity

The procedural programming followed in studing the action of ethanolic extract against the bacterial infections caused by 2-gram (+ve) and 3- gram (-ve) bacterial as described in Table-1.

Out of 05 bacteria tested on, only 03 bacteria (Anne, 1958, Klohs, *et al.* 1954 and Machillamy, 1963) [1, 4, 5] were found

susceptible, the order being *Klebsiella pneumoniae* > *Staphylococcus (local)* > *Bacillus subtilis*. The bacterial infections caused by the bacterias (Anne, 1958, Klohs, *et al.* 1954 and Machillamy, 1963) [1, 4, 5] appear to be treatable using ethanolic extract of *Rauwolfia serpentina*. Out of the various possible mechanism of action, the only possibility appears to be the inactivation of the enzymes which play a main role in the bacterial activation.

Antioxidant Activity

Preliminary studies medicated the reduction of Fe^{3+} FRAP reagent to Fe^{2+}. The tested concentration was raised from 50-5000(µg). The data showed the continuous increase in the FRAP value till 141.0 µm after which the FRAP value assumed nearly the constant value (180µm), showing levelling effects. The possibility of its application in a judicious manner as a dose for treating the ill effect of the overproduction of free radicals can not be ruled out. The antioxidant data is given in Table- 4. The data is represented graphically in fig.-1.

Table 1: Bacteria used in this study

S.No.	Bacterial species	Liquid medium	Solid medium	Temp. (°C)	Strain No.
1.	*Bacillus subtillis*	NB	NA	37	MTCC-12
2.	*Salmonella typhimurium*	NB	NA	37	MTCC-98
3.	*Pseudomonas aeruginosa*	NB	NA	37	MTCC-1036
4.	*Staphylococcus (local)*	NB	NA	37	Local
5.	*Klebsiella pneumoniae*	NB	NA	37	MTCC-432

NA-Nutrient Agar, NB- Nutrient Broth, MTCC- Microbial Type Culture Collection

Table 2: Nutrient media prepared to manufacturer specifications

S. No.	Medium	Weight (grams)	Natural pH
1.	Muller Hinton Agar	38.0	7.3±0.2
2.	Nutrient Agar	28.0	7.4±0.1
3.	Nutrient Broth	13.0	7.4±0.1

Sterilization by autoclaving at 121℃ for 15 minutes, as per the manufacturer specification.

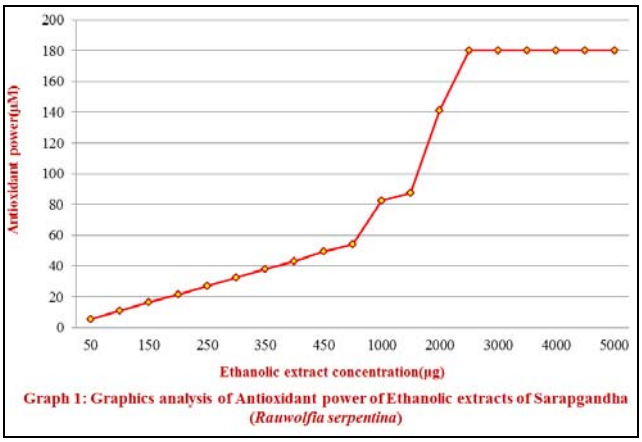
Table 3: Experimental data on Antimicrobial activity of Ethanolic extract of Sarapgandha (*Rauwolfia serpentina*) against gram (+ve) and gram (-ve) bacteria

S.No.	Strain used	Extract Concentration	Zone of inhibition (mm.)	Control
1.	*Bacillus subtillis*	0.5	10	NiL
2.	*Salmonella typhimurium*	0.5	NiL	NiL
3.	*Pseudomonas aeruginosa*	0.5	NiL	NiL
4.	*Staphylococcus (local)*	0.5	11	NiL
5.	*Klebsiella pneumoniae*	0.5	15	NiL

Diameter of well= 9 mm. Control= DMSO (Dimethyl sulphoxide)

Table 4: Experimental data on Antioxidant power of Ethanolic extracts of Sarapgandha (*Rauwolfia serpentina*)

S.No.	Ethanolic extract concentration(µg)	Antioxidant power(µM)
1.	50	5.40
2.	100	10.80
3.	150	16.20
4.	200	21.60
5.	250	27.00
6.	300	32.40
7.	350	37.80
8.	400	43.20
9.	450	49.50
10.	500	54.0
11.	1000	82.5
12.	1500	87.6
13.	2000	141.1
14.	2500	180.0
15.	3000	180.1
16.	3500	180.1
17.	4000	180.1
18.	4500	180.1
19.	5000	180.1



Graph 1: Graphics analysis of Antioxidant power of Ethanolic extracts of Sarapgandha (*Rauwolfia serpentina*)

References

1. Anne Pickering T, Wei-Wei Wang, Laine Klerry E. West J Med. 1958; 89(1):1-6.
2. Klohs MW, Draper MD, Keller FJ. Am Chem Soc. 1954; 76(10):2843.
3. Kleh J, Fazekas JF. J Am Geriatr Soc. 1956; 4(1):18-23.
4. Dhawan, Bhargava, Arora, Madan. Ind J Pharm. 1955; 18:247.
5. Macphillamy HB. Pl Sci Bull. 1963, 9(2).

6. Chakravarti, Mukherjee. ibid Sci and Cult. 1952; 3(18):338.
7. Essellier AF, Goerre J, Wegmann A. Schweiz Med Wochenschr. 1955; 85(34):808-811.
8. Bohner E, Theobald H. Disch Med Wochenschr. 1955; 80(24):929-930.
9. Jacques Genest, Lidia Adamkiewicr, Raymond Robillard, Gilles Tremblay. J Am Chem Soc. 1959; 72(7):483-489.
10. Raynaud R, Deshougus JR. Alger Medicale. 1955; 59(1):49-54.
11. Sukhdev. Env Heal Persp. 1999; 107(10):783-789.
12. Saland G, Fischer I, Hernried HP, Stoliar ED and State NY. J Med. 1956; 56(8):1276-1278.