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Electrochemical study of chemical constituents of *Rauwolfia serpentina*

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Abstract
The electrochemical study of herbal xerophytic plants of Apocynaceae family have been studied *in vivo* and vitro conditions with three electrode pairs in three different seasons. The electrical activity in living cells was determined by measuring their bio-electrode potential regulating several vital physiological processes and also maintaining the photo-electron transport activity in plants. The effect of catalyst ($CuSO_4$), respiratory substrate $C_6H_{12}O_6$ and primary salts have been studied. The origin of bio-electrode potential (BEP) developed due to formation of charge transfer complex, depends upon the availability of ions permeable at surface cuticle of the leaf. Various graphs were plotted to co-relate the results. The analysis of chlorophylls, amino acids etc. have also been made and activity was discussed.

Keywords: Vital, charge transfer, aptly, exploitation, bio-mass

1. Introduction
The subject of electrochemistry is of major technical importance as it enables the ionic reactions occurring at electrode surface in contact with an electrolyte. The electrochemical cell offers possibility for efficient production of electrical energy from chemical surfaces. The recent age is aptly the age of electricity. The reserve of fossil's fuel is limited and their unplanned exploitation in the field of energy production has poses a threat to society, to meet out the growing demand of energy. The day is not for away, when extinct of natural wealth in future would shed a shadow of energy crisis. Under such a circumstances, the only everlasting non-conventional source of energy from xerophytic plants is left as an alternative besides solar energy.

The cells of almost all plants are excitable stimuli such as, chilling, heating, cutting touching or change in external as morality result in action potential [1]. The BEP originates from various sources due to the existence of movements of free ions through membranes permeability [2] by the activity of energy consuming ion pumps embodied in most plant cells. The living biological system generates in very small amount of bio-electricity [3] through enzyme catalysed reaction working on redox principles for their body organization. The process involved like photosynthesis [4], respiration, injury and healing are electrical in nature.

Recently Jain *et al.* [5, 6] (1987,88) initiated the work on the exploitation of bio-mass energy from the plant's leaves of *Sansevieria trifasciata* with C-Mg electrode pair containing a number of bio-chemicals, like amino acids etc. and succeeded in developing leave's batteries and a bio-emf device capable of forming charge transfer complexes [7]. Similar work have also been reported earlier by a couple of workers- Sarabhai *et al.* [8] (2007) and Saket *et al.* [9] (2010) who extracted bio-electrical energy from bio-systems non conventionally. Inspired from above text, we report hither to unreported results of electrochemical study of *Rauwolfia serpentina* herbal plants.

2. Experimental
Rauwolfia serpentina is a coloycal herbal xerophytic medicinal plant, belongs to Apocynaceae family possesses a lucrative quantity of ions, with large uniform surface area. The three varieties of this system abbreviated RS_1, RS_2 and RS_3 have been studied electrochemically employing three pairs of electrodes Ag-Zn, C-Zn, and Cu-Zn in three different seasons respectively. The fresh leaf is plucked, washed and processed, after scrapping over both sides epidermis to get surface contacts. It is cut into size of 2×2 cms dimension of electrodes. The needle like fine electrodes were also prepared having the dimeter of 1 to 2 mm.

The electrodes were washed with distilled water and finally cleaned with acetone. The dried electrodes were then polished with emery paper with successive increasing fineness. The bio-emf cell is constructed by sandwiching the injured leaf between two electrodes *in vitro* condition pressed by non-conducting pressure clips.

All the electrical measurements have been made through digital panel meter (Unit-T, model DT 830 B) having the resolution of 1 mV, 0.1 µA, 1 µA and µA or with an accuracy of + 0.1% D.C. current.

The BEP has been determined several times under normal atmospheric condition during morning (6.00 AM), noon (12.00 noon) evening (6.00 PM), and midnight (12.00 PM) hours in rainy (R) winter (W) and Summer (S) seasons only. The effect of injury of leaf and the time duration of potential drop during the gradual death of plants tissues have been recorded. The pH, conductivity of biomass of sap etc. have been directly measured by digital pH and conductivity meters. The size of leaf piece measured and the number of cells in bio-mass is counted with the help of research microscope. The pigments and free amino acids were identified chromatographically by measuring their R_f values. The statistical analysis have also been made by computers.

3. Results and Discussion

The highest BEP for uninjured plants of RS_1 1354 mV (S), 1205 mV (R) and 1070 mV (W) *in vivo* condition was observed in night and lowest for RS_3 994 mV (S), 820 mV (R) and 830 mV (W) in noon and morning respectively. This might be due to stiff epidermis created obstruction for movement of ions towards electrodes due to formation of thin multi- layers. The highest potential development in injured plants for RS_1 1085 mV (S), 1020 mV (R), 1081 mV (W) in night and lowest was found for RS_3 950 mV (S), 855 mV (R) and 845 mV (W) in morning under vitro condition (Tables: 1–3 and Figs. 1–3). The total BEP in a span of 140 hours have been measured clearly indicate that maximum BEP was found for RS_3 1210 mV (W) with Ag-Zn electrode pair while minimum 4520 mV (S) was observed with Cu-Zn electrode pair. The electrolyte present in system's electron interface contribute some electrons along with the electrons donated from redox reaction consequently maximum potential developed in 50 hours for RS_2 (890 mV) and minimum was exhibited by RS (390 mV) with Ag-Zn electrode pair in summer season.

The catalyst $CuSO_4$ shows de-activating effect over the physiological activities of the tissues which reduces the potential as well as life of BED. The respiratory substrate $C_6H_{12}O_6$ delivered more charges within a duration of 50 hours, improved BED performance, also established the role of respiration in the operational mechanism of BED. The maximum potential developed for RS_2 (1170 mV) and minimum (530 mV) in the same duration with Ag-Zn electrode pair in summer season. The effect of monovalent cations (Na^+ and K^+) and anions (Cl^-) more rapidly enter cells than divalent and trivalent ions, showed high voltaic output, as they are being absorbed by the system providing additional ionic contribution in charge transfer reaction.

The loss of electrons from chlorophyll during photic excitation, causes energy conversion into ATP and reduction of NADP to NADPH and H^+ ion. The swelling and shrinking of chloroplast has been observed during photosynthesis accompanying electron flow and ATP synthesis may be electrochemical in nature resulting from a difference in [H^+], develops electrical potential across the membrane [12]. The Zwitterions of protein in the sap is fully responsible for performing redox process for flow of current. The older cells hinder the physiological process, and reduce the electrical conductance.

The plants contains fuel cells which are responsible for production of potential as it forms uni-and multi-layers of oppositely charged ions between electrode and biomass (sap) as an electrical double layers during adsorption.

The observed activity found in terms of potential is in the following order:

$RS_1 > RS_2 > RS_3$

The injured leaves give high potential in compared to uninjured leaves. In injured leaves there is no obstruction of cuticle for free movement of ions towards the site of injury for healing process and thus higher potential is created. So long as tissues are there, fuel cells give BEP. Detached leaf loose its supply of water, the tissues has to fight for maintenance of life activities, causes shivering effect over potential drainage. The chemical reaction speeded up by heating and slowed down by cooling. The fluctuation in charge potential is highest in rainy season whereas lowest in summer season i.e. transport phenomenon increases in rainy season while at high temperature more transpiration takes place, so the sap material of leaf which contains ions also get dry. In morning, photosynthesis starts and mesophyll cell of leaf get activated. In winter season, there is maximum potential at noon whereas minimum at morning, because low temperature at morning and midnight the ions present in it, get inactivated. The H_2O has the greatest conductivity of all liquids and rushing of ion conduct the potential. The consumption of oxygen has direct effect on the enhancement of power output.

The Ag-Zn electrodes pair give maximum potential. The tendency of Ag to gain the electron from bio-mass is more pronounced than Cu and others. When bio-system is suddenly killed or de-activated it, achieves the thermodynamic equilibrium and null potential is observed asymptotically under such condition, the number of tissues is very much sensitive to ambient atmosphere. The rains make the transport phenomenon more pronounced which is responsible for high BEP. The moisture may probably results in enormous reduction of internal resistance.

Table 1: Potential time duration of system

System : *Rauwolfia serpentina* (RS_1)
Electrode pairs : Cu-Zn, Ag-Zn, C-Zn,
Season : Summer
Temperature : 308 K

S. No.	Time (Hours)	Electrode pair		
		Ag-Zn	C-Zn	Cu-Zn
		Potential (mV)	Potential (mV)	Potential (mV)
1.	0	930	815	805
2.	10	945	850	795
3.	20	780	805	705
4.	30	805	780	690
5.	40	770	705	560
6.	50	730	650	420
7.	60	580	495	360
8.	70	650	505	305
9.	80	530	480	265
10.	90	435	370	130
11.	100	230	105	105
12.	110	240	70	40
13.	120	105	40	30
14.	130	50	30	20
15.	140	10	5	0
16.	150	04	0	0
17.	160	0	0	0

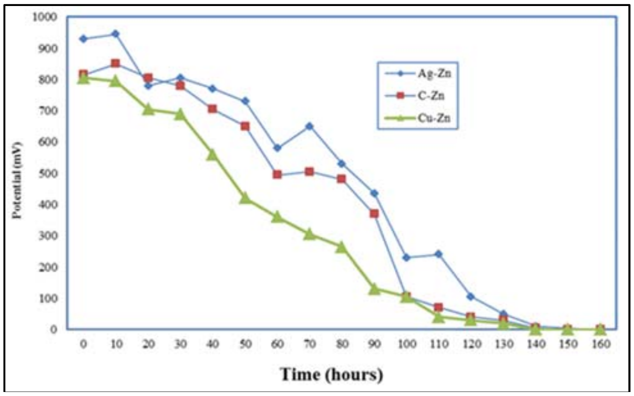

Fig 1: Potential time duration of system *Rauwolfia serpentina* (RS_1)

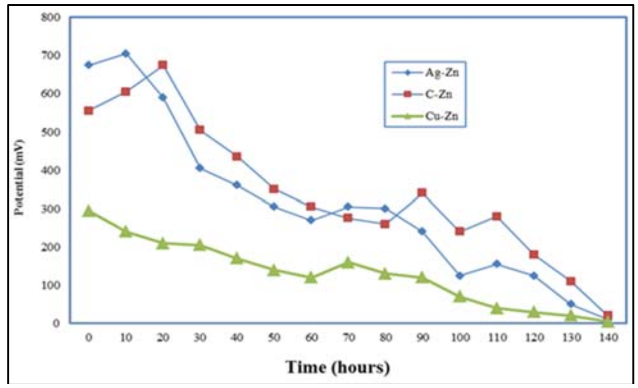

Fig 2: Potential time duration of system Rauwolfia serpentine (RS_2)

Table 2: Potential time duration of system

System : *Rauwolfia serpentina* (RS_2)
Electrode pairs : Cu-Zn, Ag-Zn, C-Zn
Season : Rainy
Temperature : 302 K

S. No.	Time (Hours)	Electrode pair		
		Ag-Zn	C-Zn	Cu-Zn
		Potential (mV)	Potential (mV)	Potential (mV)
1.	0	675	555	295
2.	10	705	605	240
3.	20	590	675	210
4.	30	405	505	205
5.	40	360	435	170
6.	50	305	350	140
7.	60	270	305	120
8.	70	305	275	160
9.	80	300	260	130
10.	90	240	340	120
11.	100	125	240	70
12.	110	155	280	40
13.	120	125	180	30
14.	130	50	110	20
15.	140	10	20	05

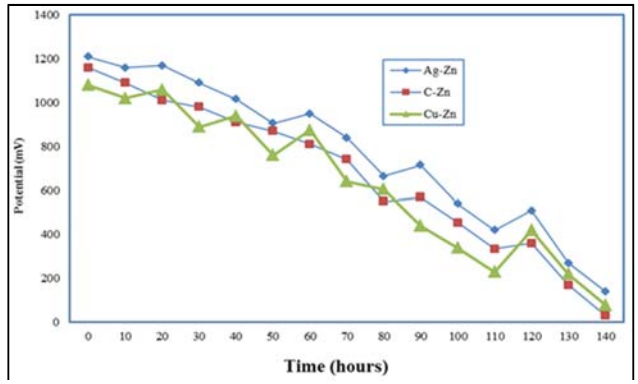

Fig 3: Potential time duration of system Rauwolfia serpentine (RS_3)

Table 3: Potential time duration of system

System : *Rauwolfia serpentina* (RS_3)
Electrode pairs : Cu-Zn, Ag-Zn, C-Zn
Season : Winter
Temp. : 288 K

S. No.	Time (Hours)	Electrode pair		
		Ag-Zn	C-Zn	Cu-Zn
		Potential (mV)	Potential (mV)	Potential (mV)
1.	0	1210	1160	1080
2.	10	1160	1090	1020
3.	20	1170	1010	1060
4.	30	1090	980	890
5.	40	1015	910	940
6.	50	905	870	760
7.	60	950	810	875
8.	70	840	740	640
9.	80	665	550	605
10.	90	715	570	440
11.	100	540	455	340
12.	110	420	335	230
13.	120	510	360	420
14.	130	270	170	220
15.	140	140	30	80

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