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A Comparative Study of Phytochemical and Biochemicals of Micropropagated and Wild *Rhazya stricta* Decne

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Abstract: Wild plants are creation of nature which are source of food, fuel,shelter,fibers,gum,andherbal medicine. Present study was carried out to investigate the biochemicals and phytochemicals of wild and micropropagated *Rhazya stricta* plant. The present investigation was aimed to promote the benefits of wild plants. For this purpose, *in-vitro* micropropagation of *Rhazya stricta* (family Apocynaceae) was successfully achieved. 20% extract was prepared in acetone, ethanol and water solvents from stem, root, fruit and leaf of *Rhazya stricta*(collected from Jamshoro district) and root, stem and leaves from micropropagated plant which were investigated to analyze the phytochemical and biochemicals. Result showed that highest anti-oxidants were observed from acetone extract of leaves (*in-vivo*) as 13.60mg/ml, flavonoids form ethanol extract of fruit as 1.98mg/ml, total phenolics from ethanol extract of leaves (*in-vivo*) as 0.84mg/ml, tannin from acetone extract of stem(*in-vitro*) as 0.61mg/ml, reducing power from ethanol extract of stem (*in-vitro*) as 1.82mg/ml, protein from acetone extract of leaves (*in-vivo*) as 9.91mg/ml, total sugar from acetone extract of leaves (*in-vitro*) as 8.00mg/ml and reducing sugar from ethanol extract of leaves(*in-vitro*) as 0.99mg/ml were found from under investigation plants.

Keywords: *Rhazya stricta*, Micropropagation, Phytochemicals, Biochemicals

1. INTRODUCTION

Wild plants are source of attraction as they protect the humans from various diseases (Chakraborty, *et al*., 2002). The eighty percent world population is dependent on herbal medicine (Fransworth, *et al*., 1985). Locally plants including their parts have been used as remedies for various ailments (Sowjanya, *et al*., 2014). The occurring of phytochemical activitiesin wild plants are the essential ingredients, which are responsible for the protection from different diseases. The wild plants contains free radicals, scavenging molecules such as phenolic compounds, vitamins, terpenoids etc. (Larson, 1988, Shahidi, *et al*., 1996). *Rhazya stricta* Decne (family Apocynaceae) is commonly found in Pakistan, in Sindh locally called as "Seenhar". It is small evergreen shrub, glabrous, upright, and poisonous (Nazimuddin and Qaiser, 1983**),** used as a traditional medication to cure relatively several illnesses (Hooper, 1906; Rahman, *et al*., 1989). *Rhazya stricta* Decne is the plant which has the qualities to minimize the effect of cancer and bacteria (Gilani, *et al*., 2007). It is also used by a tribe of Selimanial in Iran, Middle East and Karman to heal bruises quickly (Khaksari, *et al*., 2000). The leaves are added in various medications to recover pain in throat, arthritis pain, general frailty and temperature (Adam, 1998). Moreover the pod and leaves are also the part of medication used as remedy for the spots and burning purpose (Bashir, *et al*., 1994; Qureshi, *et al*. 2007). Furthermore phytochemicals possessed in *Rhazya stricta* are resource for remedy of various illnesses (Lanjwani, *et al.*, 2018).

The purpose of this investigation was topromote theadvantages and evaluate phytochemical and biochemical properties of *Rhazyastricta* (*in-vivo* and *in-vitro*).

2. MATERIALS AND METHODS

Collection of specimen

Samples for study werecollected from AmariDistrict Jamshoro, Sindh, Pakistan and washed carefully with tap water then by D.H_2O to remove superficial dust, thenwater was removed using blotting paper, carefully separated fruit/inflorescence, leaf, stem and rootthendried under shade at room temperature for 5 weeks. After that, dried material waspowdered in a grinder separatelyand kept in glass jars.

Extract Preparation

The 20 percentextractwas prepared from different parts such as fruit/ inflorescence, leaf, stem and root of wild *R.stricta* plant their roots leaf and stem of micro propagated *Rhazya stricta* in acetone, ethanol and water solvents separately. The 5.0 grams powder of each material was taken in a mortar. About1.0g of glass powder was added for fraction. Adequate amount of

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solvent was added and thoroughly rubbed with a mortar to prepare a viscous paste, transferred to falcon tubes, and centrifuged, speed up to 6000rpmfor20 minutes. After centrifugationtheextractwas filtered through Whatman filter paper No. 1, stored in sterile air tight glassbottle, andthe extraction process repeated. Finally extract volume raised to 25ml by addingthe solvents (acetone, ethanol or water) to prepare 20% extracts (Naqvi, *et al*., 2011).

Micro propagation

The seed explants of *Rhazya stricta*Decne were washed carefully with tap water, for removing the superficial dusty particles (David, *et al*., 2015), then rinsed in $D.H_2O$, followed by sodium hypochlorite (NaClO) 30%, with1-2 drop of mercuric chloride ($HgCl_2$), 2 drops tween 20 ($C_{58}H_{114}O_{26}$) on magnetic stirrer (MS300HS).Thesolution was discarded and washed three times (10 minute per wash) with distilled ($D.H_2O$). The explants were cultured individually in MS medium, added with Plant Growth Regulators (PGRs)concentration **(Table 1)**, pH adjusted 5.8±0.1, kept in the growth room, contained 16hoursday light and temperaturewasmaintained at 25±2ºC. The microshoots were sub-cultured on regular basis with the gape of twenty days in freshly prepared media with similar concentration of PGRs. For the formation of roots the microshoots were implanted in rooting media **(Table 2).** The successful rooted explants were removed from media and shifted into nursery bags containing sterile soil, covered with polythene bags and incubated in 25±2°C under 14 hours photoperiod with white and cool 3000 lux light intensity. After 21days, polythene bags were removed, rooted explants were transplanted into pots containinggarden soil, and placed in green house for acclimatization.

Quantification of Antioxidants

Total antioxidants activity of sample extracts was estimated following method reported by Prieto, *et al*., (1999). Accurately 0.2 ml test samples (T.S) were added with 2ml solution containing 0.6Msulphuric acid (H_2SO_4), 28m Msodium phosphate (Na_3PO_4) and 4mMammonium molybdate $(NH_4)_6Mo_7O_{24}$ in the test tube, by capping with aluminum foil transferred in water bath (boiling) for one and half an hour at 95ºC. Then reaction mixture was cooled to room temperature and absorbance was taken againstblank at 695 nm.

Quantification for Flavonoids

Accurately 0.1ml of TS was added in 0.3 ml of 5% $NaNO_3$in test tube for 5minutes. After that 0.3 ml of $AlCl_3$was also added again for 5minutes and then2 ml (1M) NaOH was addedthen thevolume wasraised up to 10 ml by distilled ($D.H_2O$).The blank was prepared by adding $D.H_2O$ along with all reagents (except T.S). The absorbance was read against blank at 510 nm as reportedbyDjeridane, *et al*., (2006).

Quantification of Phenolic Compounds

0.2 ml T.S was taken in a testtube and then 1.0ml of 10 fold Folin-Ciocalteudiluted and 0.8ml $Na_2CO_3$was added, mixed thoroughly; the mixture was left for half an hour at the temperature of room. The absorbance was read against the blank at 765nm as reported by Yasoubi, *et al*., (2007).

Quantification of Tanninscontent

In 0.1ml T.S 7.5ml $D.H_2O$ and 0.5ml FolinCiocalteu (1:1 v/v) reagent were added. Then 1.0 ml Na_2CO_3 (35%) was added and raised up to 10 ml volume by $D.H_2O$ thoroughly and wait for 30 minutes. The absorbance was measured at 725 nm against blank (Tamilselvi, *et al*., 2012).

Reducing Power Activity

In 1ml of T.S, 2.5 ml (0.2 M) phosphate buffer solution (pH 6.6), and 2.5 ml (1%) potassium ferricyanide K_3 [Fe $(CN)_6$] were added,mixed thoroughly, the mixture wasincubated at 50°Cup to 20 minutes. Then the reaction was terminated by adding 2.5ml trichloroacetic acid (TCA) [C (Cl_3) COOH]. Then sample was centrifuged at 3000 rpm for 15 minutes. After that, 2.5mlwas taken from upper layer in a test, then 0.5 ml (0.1%) $FeCl_3$ and 2.5 ml $D.H_2O$ was added and shook thoroughly. The absorbance was measured at 700 nm against the blank.

Quantification of total Proteins

For the quantification of total protein, (Lowry, *et al*., 1951) method was used. In a test tube 0.5 ml T.S was taken, added 2.5 ml reagent A (2% Na_2CO_3 in 0.1 N NaOH) and mixed thoroughly at Reaction time (RT). After ten minutes, 0.25ml diluted Follin-Ciocalteu (1:1 v/v) reagent with $D.H_2O$ was added and kept the reaction mixture at room temperature for 30 min to complete the reaction that developed bluish colour. The absorbance was read at 750nm against blank.

Quantification of total Sugars

Quantification of total sugar wasdetermined by Montgomery (1961) method. 0.5ml T.S was added in 2.5ml concentrated H_2SO_4 and 50µl of 80% phenol solution one by one in test tube, mixed thoroughly and wait at room temperature for 15 min. The absorbance was measured by UV-visible spectrophotometer at 485nm against blank.

Determination of Reducing Sugars

In a test tube, 2.0 ml T.S was mixed with 2.0ml freshly prepared3, 5-Dinitrosalicylic acid DNS ($C_7H_4N_2O_7$) solution andincubated in a boiling water bath for five minutes**,**wait until temperature reached to

normal. The color intensity was read against blank by spectrophotometer at 540nm as reported by Miller (1959).

3. RESULTS AND DISCUSSION

Study revealed that *Rhazya stricta*possessed a rich source of phytochemicals and biochemicals, such as antioxidant flavonoids, total phenolics, tannins, reducing power, total protein, total sugar and reducing sugars in all parts which as shown in tables3-5.

The micropropagation is very useful for mass production, desirable characters, conservation of endangered species and seasonal variations (Adel, *et al.,* 2012). In present study micropropagation of *Rhazya stricta* Decne by using seed explants in MS media supplemented with different concentration of plants growth regulators (PGR) as Kinetin, BAP and IAA for shooting formation, IBA and IAA for rooting formation was successfully achieved **(Table 1 and 2).**

During micropropagation sterilization problems was faced, the same problem was reported by Perez and Burg 2000; Leal, *et al.,* 2007. The explants of *Rhazya stricta* Decne were sterilized with 20% commercial chloride for 7 to 10 minutes (Adel, *et al.,* 2012; Mangrio, 2018) optimized the explants of *R.mucronata* with 58% commercial bleach in 20 minutes. In present study the sterilization of seed explants were optimized with 30% sodium hypochloride, two drops of tween twenty in 15 minutes time duration.

Previously, it was reported that BAP is efficient hormone for growth of *Chlorophytum borivilianum* (Sharma and Mohan 2006). The best result of micro shoot proliferation of Papaya and Strawberry was achieved in MS media fortified with Kinetin 0.5 mg/L, IBA 1.5 mg/L (Cononer and Litz 1978; Bhatt and Dhar, 2000). BAP 2.0 mg/L, Kinetin 1.0 mg/L was considered as optimal concentration for shoot multiplication of *Rhazya stricta* Decne (Adel, *et al.,* 2012). In agreement with these reports current study reveals 80% micro shoot formation, 16.8± 0.05 microshoots per explant and 8.8±0.04 cm average micro shoot length in 35 days were achieved when explants were implanted in M.S media supplemented with different concentration and combination of Kinetin 2.0, BAP 1.0, IAA 0.5 mg/L growth regulators (Table 1).

The successful regenerated micro shoots were transferred into rooting media without or with combination of different plant growth regulators. The obtained result showed significantly variable response of MS media in different combinations and concentration of IBA and IAA as 10 to 88% microshoots were commenced into root formation. Comparatively the response of IBA is more effective in root than IAA. The efficacy of IBA during in vitro root formation was also reported by (Shahzad, *et al.,* 2009; Ahmed, *et al*., 2013; Faisal, *et al.,* 2014). In current study the maximum root formation response as 88% microshoots developed into roots with 4.2 number of roots per microshoot and 5.9 cm average length of roots were observed when microshoots were incubated in MS media additionally supplemented with IBA 2.5 mg /L, and IAA 0.5 mg/L PGR. Furthermore it was also analyzed that micro shoots failed to develop into the roots incubated in MS media without any addition of PGR. **(Table 2).** Whereas, (Adel, *et al.,* 2012) from *Rhazya stricta* Decne, Mangrio (2018) in *Avicenna marina* reported the root formation with combined action of IBA and IAA. The current investigation almost agrees with these reports.

Phytochemicals and bio chemicals are the essential compounds existed in wild plants which used as medicines (Srivastav, *et al*., 2011) against cancer, oxidants, inflammation, diarrhea, stomach and radio injuries, constipation, burning, wound and skin diseases (Sharma, *et al.,* 2014). Oxygen is necessary for living purpose because it oxidize the food to produce energy. During the process so many harmful molecules are also produced called as free radical, in turns chains of chemical reaction starts which cause death and damage of vital cells of the body. Anti-oxidantsinhibits the chain of these reactions by removing the free radicals from the body before the death and damage of vital body cells (Sethi and Sharma 2011). In previous investigations many researchers reported the presence of phyto and biochemicals components from *Rhazyastricta, (*Bhanger, *et al*., 2006) extracted antioxidants from leaves of *Rhazya stricta* Decne in methanol extract. Lanjwani, *et al.,* (2018) extractedthehigh percentageof antioxidants, phenolics and proteins from the leaves of *Rhazya stricta* similar in present investigation. The highest amount of antioxidantswas observed in leaves acetone extract from natural growing *Rhazya stricta* as 13.60mg/ml.The highest flavonoids was observed from ethanol extract of fruit as 1.98mg/ml. The highest amount of phenolics from the ethanol extract of leaves as 0.84mg/ml. Similar findings were reported by Lanjwani, *et al.,* (2018). The maximum tannins content were quantified from acetone extract of stem as 0.6mg/ml. The highest reducing power activity was observed in ethanol extract of stem as 1.82mg/ml from micro propagated *Rhazyastricta*, total protein from acetone extract of leaves, total sugars and reducing sugars were increased in micropropagated*Rhazya stricta* in acetone and ethanol extract of leaves as 8.00mg/ml and 0.9mg/ml respectively. It is also observed that antioxidants, flavonoids, phenolics and total protein were increased in *in-vivoRhazyastricta*,whereas the tannins, reducing power, total sugars and reducing sugars were increased in micropropagated *Rhazyastricta* **(Tables3-5).**

Table-1. Effect of different concentrations and combination of PGR (Kin, BAP and IAA) on microshoot formation in *Rhazya stricta*

MS Medium with PGRs (mg/L)			Explants % formed microshoots	No of microshoots per Explant (Mean±SD)	Microshoots length in cm after 35 days (Mean±SD)
Kin	BAP	IAA			
0.0	0.0	0.0	0.0	0.0	0.0
0.0	0.5	0.0	13	3.0±0.2	4.7±0.2
0.0	1.0	0.0	29	6.0±0.05	4.9±1.1
0.0	1.5	0.0	32	6.2±0.09	5.3±0.5
0.0	2.0	0.0	36	6.6±0.1	5.1±0.4
0.0	2.5	0.0	42	9.1±0.05	6.2±1.1
0.5	0.0	0.0	17	3.7±0.05	6.6±1.2
1.0	0.0	0.0	26	5.9±0.1	5.7±0.5
1.5	0.0	0.0	29	6.1±0.09	6.8±1.2
2.0	0.0	0.0	39	5.9±0.05	7.0±1.5
2.5	0.0	0.0	42	9.3±0.2	7.6±1.0
0.0	0.0	0.5	10	2.9±0.08	4.0±1.2
0.0	0.0	1.0	20	4.4±0.05	4.8±0.5
0.0	0.0	1.5	22	4.6±0.1	4.7±1.2
0.0	0.0	2.0	19	4.1±0.05	4.2±1.1
0.0	0.0	2.5	30	6.8±0.09	5.5±0.5
1.0	1.0	1.0	67	12.0±0.2	7.3±0.5
2.0	2.0	2.0	60	11.9±0.1	7.9±1.1
2.0	1.0	0.5	80	16.8±0.05	8.8±0.4
3.0	0.5	1.0	49	9.0±0.1	6.7±0.5

Table-2. Effect of different concentrations and combination of PGR on *in-vitro* root formation in *Rhazya stricta*

MS Medium with PGRs (mg/L)		Explants % formed roots	No of roots per microshoot (Mean± SD)	Rootslength in cm after 28 days (Mean± SD)
IBA	IAA			
0.0	0.0	0.0	0.0±0.0	0.0±0.0
0.5	0.0	10	1.0±0.2	2.0±0.2
1.0	0.0	24	2.0±0.05	2.8±1.1
1.5	0.0	28	2.1±0.1	2.9±0.5
2.0	0.0	33	2.0±0.05	2.7±0.4
2.5	0.0	55	3.0±0.05	2.9±1.1
3.0	0.0	71	3.2±0.1	4.1±1.2
0.0	0.5	18	1.0±0.09	1.9±0.5
0.0	1.0	22	2.0±0.05	2.6±1.2
0.0	1.5	25	2.1±0.2	2.5±1.5
0.0	2.0	28	2.2±0.08	2.9±1.0
0.0	2.5	26	2.1±0.05	2.1±1.2
1.0	0.5	30	2.4±0.1	2.2±0.5
1.5	1.0	41	2.5±0.05	2.6±1.2
2.0	1.5	68	3.0±0.09	3.9±1.1
2.5	1.0	66	3.1±0.2	4.8±0.5
3.0	1.5	50	3.0±0.1	4.9±0.5
2.5	0.5	88	4.2±0.05	5.9±1.1
3.5	0.5	77	4.1±0.05	5.8±0.4

Table-3: Phytochemical and biochemical contents in different parts of Rhazya stricta in acetone solvent (mg/ml)

Test		Parts			
		Root	Stem	Leaves	Fruit
Antioxidants	*	7.13±0.1	11.24±0.2	13.6±0.09	8.32±0.01
	**	6.97±1	10.81±0.2	14.1±0.5	-
Flavonoids	*	0.83±0.01	1.02±0.01	1.42±0.02	1.56±0.02
	**	0.56±0.03	0.49±0.01	0.79±0.04	-
Total phenolics	*	0.33±0.05	0.54±0.04	0.42±0.05	0.35±0.04
	**	0.41±0.05	0.51±0.05	0.48±0.05	-
Tannins	*	0.15±0.02	0.28±0.02	0.37±0.01	0.35±0.03
	**	0.31±0.02	0.61±0.61	0.58±0.04	-
Reducing power	*	0.05±0.2	0.07±0.24	0.28±0.33	0.12±0.26
	**	0.89±0.04	1.09±0.02	1.22±0.03	-
Protein	*	8.18±0.06	8.18±0.01	9.91±0.03	8.91±0.01
	**	8.1±0.64	8.01±0.5	9.03±0.5	-
Total sugars	*	1.77±1.77	2.18±0.03	3.59±0.03	2.78±0.11
	**	2.11±0.5	3.18±0.39	2.99±0.32	-
Reducing sugars	*	0.55±0.02	0.56±0.01	0.58±0.02	0.55±0.01
	**	0.49±0.05	0.61±0.06	0.28±0.02	-
* =*In-vivo*, **=*In-vitro*, - = not analyzed					

Table-4: Phytochemical and biochemical contents in different parts of *Rhazya stricta* in ethanol solvent (mg/ml)

Test		Parts			
		Root	Stem	Leaves	Fruit
Antioxidants	*	5.49±0.05	5.67±0.06	6.69±0.06	4.37±0.7
	**	4.28±0.05	4.95±0.07	7.26±0.06	-
Flavonoids	*	0.71±0.02	1.26±0.02	1.7±0.02	1.98±0.02
	**	0.36±0.02	0.32±0.01	0.98±0.03	-
Total phenolics	*	0.58±0.08	0.68±0.03	0.84±0.15	0.82±0.14
	**	0.63±0.08	0.79±0.03	0.99±0.11	-
Tannins	*	0.23±0.03	0.33±0.05	0.36±0.02	0.35±0.02
	**	0.41±0.03	0.28±0.05	0.62±0.2	-
Reducing power	*	1.36±0.36	1.49±0.34	1.44±0.32	1.55±0.34
	**	1.52±0.04	1.82±0.04	1.71±0.02	-
Protein	*	2±0.01	2.04±0.01	2.02±0.01	2.05±0.01
	**	3±0.7	2.99±0.6	3.88±0.6	-
Total sugars	*	5.29±0.7	6.63±0.07	7.8±0.05	5.42±0.02
	**	5.99±0.77	6.88±0.6	6.8±0.68	-
Reducing sugars	*	0.51±0.01	0.55±0.02	0.57±0.02	0.5±0.02
	**	0.42±0.01	0.6±0.03	0.99±0.08	-
*=*In-vivo*, **=*In- vitro*, - = not analyzed					

Table-5: Phytochemical and biochemical contents in different parts of *Rhazya stricta* in water solvent (mg/ml)

Test		Parts			
		Root	Stem	Leaves	Fruit
Antioxidants	*	5.91±0.04	6.67±0.04	2.01±0.04	2.62±0.01
	**	6.08±0.04	5.87±0.05	3.84±0.04	-
Flavonoids	*	1.21±0.02	0.7±0.01	1.28±0.02	0.84±0.02
	**	0.71±0.04	0.81±0.02	0.66±0.66	-
Total phenolics	*	0.48±0.09	0.57±0.1	0.77±0.04	0.78±0.11
	**	0.41±0.1	0.61±0.11	0.66±0.04	-
Tannins	*	0.09±0.02	0.22±0.02	0.31±0.01	0.28±0.02
	**	0.28±0.02	0.42±0.02	0.49±0.01	-
Reducing power	*	0.44±0.2	0.55±0.24	0.72±0.31	0.5±0.21
	**	1±0.1	0.62±0.04	0.87±0.01	-
Protein	*	4.55±0.09	3.66±0.04	3.84±0.04	3.83±0.08
	**	4.66±0.94	6.87±0.04	4±0.05	-
Total sugars	*	4.47±0.16	4.69±0.14	7.01±0.01	6.15±0.01
	**	5.23±0.06	5.2±0.47	8±0.08	-
Reducing sugars	*	0.5±0.01	0.5±0.03	0.53±0.02	0.51±0.03
	**	0.78±0.03	0.29±0.03	0.76±0.01	-
* =*In-vivo*, **=*In- vitro*, - = not analyzed					

4. CONCLUSION

Present investigation concluded that *Rhazya stricta* growing under natural conditions as well as micropropagated possessed a rich source of phytochemicals and biochemicals components in all investigated parts so the data obtained from this investigation will benefit the researchers, herbalists and pharmacists to develop the herbal, homeopathically and medicinal drugs.

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