WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 6, 445-455. Research Article ISSN 2277– 7105

EFFECT OF THIRST STRESS ON THE PRODUCTION OF PHYTOCHEMICAL CONSTITUENTS OF *RHAZYA STRICTA* PLANT AND ANALGESIC ACTIVITY OF ITS BIOACTIVE FRACTIONS

Usama Shaheen[1*,2] and Muhammad Ahmed[3]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah, 21955, Saudi Arabia.

[2]Department of Pharmacognosy, Faculty of Pharmacy, Al Azhar University, Cairo, Egypt.

[3]Department of Pharmacology, Faculty of Pharmacy, Umm Al-Qura University, Makkah, 1355, Saudi Arabia.

Article Received on
04 April 2015,

Revised on 24 April 2015,
Accepted on 20 May 2015

***Correspondence for Author**
Usama Shaheen
Department of Pharmacognosy, Faculty of Pharmacy, Umm Al-Qura University, Makkah, 21955, Saudi Arabia.

ABSTRACT

Rhazya stricta Decne is an important folkloric medicinal plant used in Saudi Arabia and other countries of Arabian Peninsula to relieve various diseases. Alkaloids and flavonoids represent the most active secondary metabolites of this plant. Rain is the only source of water for desert wild plants. This search includes the estimation of total phenol, flavonoids, and alkaloids in two plant samples. Describing the effect of prolonged thirst on the production of these constituents in the plant and assessment of the analgesic activity of its different extracts. The total phenol and flavonoid contents were estimated using spectrophotometer assay by Folin-Ciocalteau and $AlCl_3$ reagents respectively. While the concentration of its alkaloids was determined spectrophotometrically through the formation of ion-pair complex with acidic dye (methyl orange), followed by measuring the absorbance of produced color at λmax 530 nm. Also, the analgesic activity of ethanol extract, alkaloid fraction, and phenol rich fraction were determined by hot plate test. The result of these assays found that the alkaloid content was decreased in the absence of rain while the phenol and flavonoid contents were increased by thirst in a long period of no rain. The ethanol extract (20 mg/kg) and Phenol fractions (10 and 20 mg/kg) were found to exhibit significant analgesic activity in comparison with the stranded.

KEYWORDS: *Rhazya stricta*, Secondary metabolites, Thirst, Alkaloids, Flavonoids, Analgesic activity.

INTRODUCTION

Rhazya stricta Decne is an important folkloric medicinal plant used in Saudi Arabia and other countries of Arabian Peninsula. It is wildly growing throughout tropical regions all over the world especially in Gulf countries (Anira et al. 2000). It is one of the eleven plants grow in all six zones of Saudi Arabia especially in the Makkah area and forming one of the main plants of "Flora of Makkah" (Meelad, 1987).

The reported phytochemical studies conducted in various parts of *R.* stricta plant revealed the presence of over 100 alkaloids. Many of these alkaloids have been reported to have anticancer activity. (Atta-ur-Rahman and Khanum, 1987; Marwat et al. 2012), and few non-alkaloidal compounds (Zaman, 1990; Sultana and Khalid, 2010).

All parts of the plant were reported to be used in traditional medicine for various ailments. Such as diabetes, foot burning, skin diseases, stomach pain and antihypertensive (Chopra et al., 1956; Dymock et al., 1983). Khan and Khan, (2007) reported that it is used in fever, general debility and as curative for chronic rheumatism , colic, syphilis, toothache, urinary tract diseases, vermifuge, and wounds (Mukhopadhayay et al., 1983; Saeed et al., 1993). Some of its indole alkaloids showed various biological activities e.g. antitumor, antimicrobial and lowering effect on blood pressure, and stimulation of central nervous system (Verpoorte, 1998). But more work needs to be done regarding its medicinal importance (recording and exploring recipes) for finding evidence of its safety and efficacy in medicine. Therefore, such studies are recommended in various parts of the countries of Arabian Peninsula where it grows.

The literature showed the presence of more than hundred of alkaloids and some of the flavonoids with the vast array of biological activities. But there is no evidence about the amount of these valuable constituents in the plant, which is wildly distributed in the kingdom. The rainfall all over the kingdom is variable from zone to another and in Makkah from year to another. We here in the wish to investigate the effect of rainfall on the production of these constituents in this plant. There is no rain in Makkah city for 12 months (March 2011 to March 2012) except once at 3rd of October 2011 of heavy rain lasting about one hr.

MATERIALS AND METHODS

PLANT MATERIALS

A thirst sample of the plant collected in March 2012 which didn't rain for 12 months (March 2011 to March 2012) except once at 3rd of October 2011. Another normal sample was collected from the same place in March 2013 where it is rained seven to eight times most of which are medium to heavy rain. The plant materials used in this work consisted of the leaves of *Rhazya stricta* Decne collected from the desert fields of Umm Al-Qura University, Abdia, Holly Makkah, Kingdom of Saudi Arabia. The plant samples were kindly identified by Dr. Kadry N. Abd AlKhaliq, Associate Professor of plant taxonomy, Faculty of applied sciences, Umm Al-Qura University, Abdia, Holly Makkah, Kingdom of Saudi Arabia.

Materials and equipments

Thin layer chromatography (TLC): Silica gel 60 F254 (with fluorescent indicator) pre-coated sheets 20 x 20 cm, 0.2 mm (Merck). Solvent systems: The following solvent systems were applied for the TLC of crude extracts. a) n-hexane- chloroform (90 :10). b) chloroform - methanol - water (80: 20: 2). c) hexane – ethylacetate (75:25). Reagents: Vanillin/H_2SO_4 (TLC spray reagent) (Szilaggi et al., 1984). Dragendorf's reagent (TLC spray reagent) , Mayer's reagent Wagner reagent. Aluminium trichloride ($AlCl_3$) (Sigma-Aldrich USA); Folin-Ciocalteau (Sigma-Aldrich USA); Methyl orange (Sigma-Aldrich USA); Ph paper (0 - 14 range Merchyl- Nagel company). APPARATUS: Yamato Rotary evaporator RE 300, Water bath BM 510, Japan, vacuum-system. Laboratory Oven, Model 05015-52, 230 volts 800 watts LAB TECH company. U.K. UV 254/366 nm, Chromato – Vue C-70 G, Duo-UV-source For thin layer chromatography, UVP UPLAND CA 91786 U.S.A. UV-spectrophotometer: Agilent, Cary 60 single beam, Scanning, weave length range 190- 900 nm, weave length accuracy ±1nm, weave length resolution 1nm Band wide 4 nm. Agilent Technologies, Japan. Ae ADAM, 300. PGW, capacity: 300 g x 0.001 g, U.K. Digital balance :Metler Tolido , capacity 120 g, 6 digits , sensitivity 0.1 mg. Hot plate stirrer LAB TECH., 230 v 50 Hz. 2 AMP. Dinan LAB TECH. U.K.Preparation of different plant extracts for analgesic activity of normal sample of *R. stricta*.

The leaves were grounded into fine powder, and 201 g were extracted by maceration in 80% ethyl alcohol (500 ml) with continuous stirring. It is followed by filtration through Whatman filter paper no 1; the marc extracted again three times with 80% ethanol (500 ml) 24 h each time, till complete exhaustion. It was achieved by the negative test of alkaloids and

flavonoids and comparative TLC. The combined ethanol extract was collected and dried under vacuum at 40°C to afford 25 g dried extract.

Preparation of the alkaloidal and phenolic fractions

The combined ethanolic extract of *R. stricta* was suspended in distilled water (300 ml) and defatted with n-hexane. The defatted extract was basified using NH_4OH, and the P^H was adjusted to 9 using MACHEREY-NAGEL P^H paper. The aqueous layer was partitioned and extracted with chloroform till complete exhaustion that was achieved by the negative test of alkaloids using Dragendof's reagent and comparative TLC, to afford the chloroform fraction of alkaloids. While the aqueous fraction containing the flavonoids and other phenolic constituents (Phenolic fraction). The chloroform fraction of alkaloids was acidified with 5% hydrochloric acid and extracted with chloroform that takes the sterols and terpenoids leaving the alkaloids in the aqueous layer. The remaining aqueous layer was rendered alkaline using NH_4OH, and the P^H was adjusted to 9. Thin, this aqueous layer was partitioned and extracted with chloroform till complete exhaustion that was achieved by the negative test of alkaloids using Dragendof's reagent and comparative TLC, to afford the purified chloroform fraction of alkaloids. The alkaloidal and phenolic fractions were evaporated separately under vacuum 40°C to dryness and kept in the freezer until the time of biological activity.

Preparation of methanolic extract for spectrophotometric determination of total alkaloids, phenols and flavonoids in two samples of *R. stricta.* leaves collect on March 2012 and March 2013.

Ten grams powder of each sample was extracted quantitively with methanol 95 % (100 ml X 5) till complete exhaustion. A negative test for alkaloids and flavonoids to give Methanol extract of 2012 (2.1 g) and methanol extract 2013 (1.95 g). The two extracts were kept in the freezer till the beginning of a comparative spectrophotometric analysis of their alkaloids, phenol, and flavonoid content.

Estimation of the total phenol content in methanol extracts (2012 & 2013) of *R. stricta* growing in Makkah region under different rain conditions.

The concentration of total phenol of different extracts was determined using the Folin-Ciocalteau reagent. As described by Norazaidah, et al (2006). One gram of each methanol extracts (2012 & 2013), were dissolved in 10 ml methanol (80 %) to afford 100 mg / ml of each fraction. Each sample was shacked vigorously followed by filtration through filter paper (Whatman no.1.) and in the test tubes, 0.5 ml of each sample has been added followed

by 2.5 ml of 0.2 N Folin-Ciocalteau reagent and set aside for five minutes. Seven ml of Na_2CO_3 solution (7.5%) were then added. These solutions were kept at room temperature for two hours. The concentration was determined through the determination of absorbance measured at λmax 765 nm using Agilent –Cary 60 spectrophotometer. A standard calibration curve was made by gallic acid (0 - 125 mg/ml). The concentration total phenol was expressed in mg of gallic acid equivalents (GAE) / g of extract.

Estimation of the total flavonoid concentration in methanol extracts (2012 & 2013) of *R.stricta* growing in Makkah region under different rain conditions

The concentration of total flavonoid was determined using the Dowd method (Meda *et al*, 2005) with some modification, 1 g of the methanolic extracts (2012 % 2013), were dissolved in 10 ml methanol (80 %) to afford 100mg/ml of each fraction and each sample was shacked vigorously followed by filteration through filter paper (whatman no.1.). Six ml of 2 % $AlCl_3$ in methanol was mixed with 6 ml of each extract solution (100 mg/ml). The absorbance of the resulting complex was measured after 20 minutes at λ_{max} 420 nm using Agilent –Cary 60 spectrophotometer. A blank sample was made by 6 ml extract solution with 6 ml methanol free from $AlCl_3$. A standard curve was done using quercetin (0 - 62 mg/ml) as the standard. The concentration of total flavonoid expressed as mg of quercetin equivalents (QE) / g of extract.

Estimation of the total alkaloid content in methanolic extracts (2012 & 2013) of *R. stricta* growing in Makkah region under different rain conditions

The total alkaloid content was determined spectrophotometrically; this method depends on the fact that the acid dye (methyl orange) can form an ion-pair complex with the alkaloids. This method was established by Pundarikakshudu et al. (2005), and we use it with slight modification. This acidic dye forms a complex with alkaloids that selectively extracted into chloroform layer. The dye was liberated from the complex by treatment with dilute HCl, forming a pink color of methyl orange in the acidic media. The pink color of the liberated dye (methyl orange) is proportional to the amount of alkaloids, and the absorbance was measured using Agilent –Cary 60 spectrophotometer at λmax 530 nm. The method adopted for extraction of alkaloids from samples has the advantage of extraction of mainly the alkaloids and not the other interfering substances.

Preparation of solutions: Methyl orange solution (0.05%) in distilled water was prepared, Phosphate buffer solution (pH 4.6) was prepared by adjusting the pH of sodium phosphate

(5.2 g and 2.4 g glacial acetic acid in 100 ml distilled water). Brucine standard solution (1mg/ml) was made by dissolving 10 mg pure Brucine (Sigma Chemical, USA) in 10 ml chloroform and complete with methanol to final volium100 ml.

Preparation of standard curve: Brucine standard solutions were prepared with concentrations of (5, 10, 15, 20, 25 and 30 µg) and transfer 10 ml of each to different separating funnels. Then, add 5 ml pH 4.6 phosphate buffer and 3 ml methyl orange solution , the complex formed was extracted with chloroform thrice (10, 10, 5 ml). The combined chloroform extract was transferred into a separating Funnel, acidified and diluted with 25 ml of 1M hydrochloric acid, the dye librated into hydrochloric acid layer was separated, followed by measuring the absorbance at λmax 530 nm against blank. Blank was prepared by the same mentioned procedure but without brucine.

Preparation of plant samples: The dried extracts (10 mg) of *R.* stricta (2012& 2013) was dissolved in (10 ml) chloroform and then filtered. One ml (1 mg of extract) of this solution was transferred to a graduated flask and diluted to 10 ml with chloroform. Then, add 5 ml phosphate buffer and 3 ml methyl orange solution, the complex formed was extracted with chloroform thrice (10, 10, 5 ml). The combined chloroform extract was transferred into separating funnel and acidified and diluted with 25 ml of 1M hydrochloric acid. The dye librated into hydrochloric acid layer was separated, and the absorbance was measured at λmax 530 nm against blank.

Determination of analgesic activity of different extracts of a normal sample of *R. stricta* (2013).

Animals: Healthy adult mice weighing 18-30 g were used in the present study. Mice were kept maintained in polypropylene cages with six mice in each cage with food and water ad libitum, at standard temperature (20-23°C). Six mice were used in each treatment. All experiments were conducted between 9 am and 3pm. They were designated as group-I, group-II, group-III, group-IV group-V, group VI and group-VII containing six mice in each group for control (0.9% NaCl). Positive control (Paracetamol, 100 mg/kg, p.o) and test sample groups (10 and 20 mg/ kg p.o) respectively.

Hot Plate Test: The Individual animal was placed on a hot plate with fixed temperature at 55±0.5°C (Harvard Apparatus Ltd., Kent, UK). The central antinociceptive activity of paracetamol was performed by using a modified hot plate test following the method of

Lavich et al. (2005). Pini et al., 1997 suggested hot plate test as a good model to evaluate the central antinociceptive activity. After the drug administration at specific time intervals (0, 30, 60 and 120 min) the reaction time was measured for all control and treated mice. Each mouse had its own control.

Statistical analysis: All the data were shown as Mean±SEM. Data were statistically analyzed by using the one-way Analysis of Variance (ANOVA) followed by tukey test. Level of $p<0.05$ considered as significant

RESULTS AND DISCUSSION

The literature revealed the presence of more than hundred of alkaloids and some of the flavonoids with a vast array of biological activities but there is no evidence about the amount of these valuable constituents in the plant. In the extraction of alkaloids, we use the acid-base extraction method. It has a beneficial effect in case of isolation of alkaloids from other constituents especially phenolic constituents. Because of when the alkali like ammonia release the alkaloidal base rendering it water insoluble in the same time render the phenolic constituents like flavonoids and phenolics acids more soluble in water. Upon extraction with an organic solvent like chloroform takes the alkaloidal base leaving the other phenolics in the aqueous layer. Also, the uses of ammonia has an advantage over other alkalis due to the easy evaporation of the excess.

The ten grams dried powdered of each sample of 2012 & 2013 gave 2.1g and 1.95 g. methanol extract with 21 and 19.5 % extractive value respectively, this means that the constituents more concentrated in thirst sample of the plant. The comparative phytochemical screening and chromatographic studies revealed that there is no major difference in the type of constituents in the two samples of the plants, but there is a big difference in the amount. The concentration of total phenol of the methanol extracts (2012 & 2013) of *R. stricta* in gallic acid equivalents was determined by Folin & Ciocalteu's Phenol reagent. This is in vitro method for determination of phenols in natural products with some sort of false reading with reducing nonphenol substances and this is the drawback of this method (Singleton et al. 1999). Although, it is the most common method used for determination of phenols in natural products.

The highest value was obtained for *R. stricta* 2012 Methanol extract with 33.9 mg of GAE/g of extract in contrast to 27.1 mg of GAE/g of *R. stricta* 2012 methanol extract (table 1) in

contrast to 33.9 mg of GAE/g of *R. stricta* 2012 with percentage increase during thirst of 25% for production of phenolic contents.

Table 1. Total phenol, flavonoids, and alkaloids contents in the methanol extracts (2012 & 2013) of *R. stricta* growing in Makkah region under different rain conditions

Plant extract	Total phenol, Mg of GAE/g of extract	Total flavonoids, Mg of QE/g of extract	Percentage of flavonoids to total phenol content	Total alkaloids, Mg of BE/g of extract
***R. stricta* 2012 methanol extract**	33.9	22.3	65.7 %	91.2
***R. stricta* 2013, Methanol extract**	27.1	21.4	78.9 %	102.0

Flavonoids which occur both in the free state and as glycosides are the largest group of naturally occurring phenols. Flavonoids (or bioflavonoids), also collectively known as Vitamin P. The flavonoids have special interest recently because of their potential beneficial effects on human health. (Kelm et al., 2005; Schreier, 2005). The highest flavonoid concentration was present in the sample of *R. stricta* 2102 with 22.3 mg QE/g with the least concentration of *R. stricta* 2103 with a concentration of 21.4 mg QE/g. There is 4.2 % decrease in the sample of 2013 in corresponding to the sample of 2012. The total alkaloid content of the methanolic extracts (2012 & 2013) of *R. stricta* was determined using the method of Pundarikakshudu et al. (2005). The highest alkaloid content was observed in the sample of *R. stricta* 2013 with concentration of 102 mg. Brucine equivalent/ g of extract while the *R. stricta* 2012 has the 91.2 mg brucine equivalent/ g of extract (Table 1), this variation in the concentration of alkaloids describe the effect of rain on the plant by increasing the amount of alkaloids by 10.6 % in good rain year in contrast to phenol and flavonoid content. The most of alkaloids of *R. stricta* belonging to the indol alkaloids . Which is closely related to the anticancer alkaloids of vinca plants (vincristine and vinblastine) and Vincamine and its derived drug vinpocetine (Chevallier 1996; Chen et al. 1999). This plant is containing a high amount of alkaloids comparatively to other plants.

Results analgesic activity of different extracts of *R. stricta*: As shown in Table 2, the normal (pretreatment) withdrawal latency of control mice was 13.25 ± 0.62 sec. After saline injection, this value showed insignificant variation during the whole experimental period (2 hr). After injection of paracetamol, the withdrawal latency was gradually and significantly

prolonged. Starting 15 min after injection, reaching a maximum of 26.63 ± 0.86 seconds after 90 min this increase is statistically significant as compared to its control. However, it started to decline back to reach 18.25 ± 0.77 sec at the end of the evaluation period (120 min).

Table 2. Results of hot plate test

	0min			30min			60min			120min		
	Mean	**±**	**S.E**	**Mean**	**±**	**S.E**	**Mean**	**±**	**S.E**	**Mean**	**±**	**S.E**
Control	13.25	±	0.62	12.25	±	0.75	13.88	±	0.45	13	±	0.6
Paracetamol	12.13	±	0.58	23.25	±	1.15*	24.5	±	1.27*	18.25	±	0.77
ALK (10mg/kg)	14.25	±	0.74	16	±	1.58	15.75	±	0.62	14.25	±	1.75
ALK (20 mg/kg)	16.6	±	2.53	15.25	±	0.47	18	±	1.17	17.6	±	1.03
Ethanol extract (20mg/kg)	13.16	±	0.71	18.3	±	0.6*	15.6	±	1.24	14.5	±	2.26
PH (10 mg/kg)	17	±	0.8	16.6	±	1.8	19.16	±	1.65	22.2	±	1.2
PH (20 mg/kg)	17	±	0.89	27	±	1.75*	18	±	1.31	19.33	±	1.11

** indicates P<0.05* ALK= Alkaloid fraction PH = Phenol fraction

Table 2 showed the effect of the alkaloid fraction (10 and 20 mg/kg). Ethanol extract (20 mg/kg) and phenol fraction (10 and 20 mg/kg) on hot plate test. ALK (10 and 20mg/kg), were found to increase the withdrawal latency time after 30 and 60 min respectively, but this increase was statistically nonsignificant. The ethanol extract (20 mg/kg) was found to exhibit an increase in latency time, after 30min, reaching its maximum of (18.3+0.6) when compared with its control. This increase in latency time was statistically significant (P<0.05). However, after 120 min it started to decline back to normal. The present results also showed that PH (10 and 20 mg/kg) also increase the latency or reaction time, and it reached its maximum value. After 120 min and 30 min respectively, at the dose of 20 mg/kg this increase was found to be statistically significant (p<0.05).

CONCLUSIONS

This study demonstrates the effect of rain deficiency on the production of secondary metabolites of *R. stricta* plant and its analgesic activity. The stress of rain deficiency leads to increase the production of flavonoids and other phenolics in contrast to decrease in alkaloids production due to thirst stress. Phenol fractions (10 and 20 mg/kg) and ethanol extract (20 mg/kg) showed significant analgesic activity relative to paracetamol in hot plate test.

ACKNOWLEDGEMENTS

The authors would like to thank Institute of Scientific Research and Revival of Islamic Heritage at Umm Al-Qura University (Project ID 4331009) for the financial support.

REFERENCES

1. Anira M.O., M.U. B. Ali, A.K. Bashir, S. Dhanasekaran, A. D. Tibiric and L. M. Alves (2000). Mechanism of the Hypotensive Action Of *Rhazya Stricta* Leaf Extract In Rats. Pharmacological Research, 42(3): 369-378.
2. Atta-ur-Rahman, K.A. Alvi., (1987). Indole alkaloids from *Alstonia scholaris*. Phytochemistry, 26: 2139–2142.
3. Chen Y. C., Y. C. Liang, S. Y. Lin-Shiau, C. T. Ho, and J. K Lin., (1999). Inhibition of TPA-induced protein kinase C and transcription activator protein-1 binding activities by theaflavin-3, 3'-digallate from black tea in NIH3T3 cells. J Agric Food Chem., 47: 1416-1421.
4. Chevallier A., 1996. The Encyclopedia of Medicinal Plants. Dorling Kindersley, London.
5. Chopra R. N., S. L. Nayar. and Chopra, I. C., (1956). A Glossary of Indian Medicinal Plants, pp. 212. New Delhi: CSIR.
6. Dymock W. C., J. H. Warden and, H. Hooper (1983). Pharmacographia India, p. 391. London: Kegan Paul, Trench, Trubner & Co.
7. Kelm M. A., J. F. Hammerstone, and H. H. Schmitz (2005). Identification and quantitation of flavanols and proanthocyanidins in foods: how good are the datas? Clin Dev Immunol, 12: 35-41.
8. Khan S., G.M. Khan (2007). In Vitro Antifungal Activity of *Rhazya stricta* Decne. Pak. J. Pharm. Sci., 20(4): 274-279.
9. Lavich T.R, R.S. Cordeiro, P.M. Silva, M.A. Martins (2005). A novel hot-plate test sensitive to hyperalgesic stimuli and non-opioid analgesics. Braz J Med Biol Res., 38: 445-51.
10. Marwat S. K., K. Fazal-ur-Rehman, Usman, S. S. Shah,, N Anwar. and I. Ullah (2012). A review of phytochemistry, bioactivities and ethno medicinal uses of *Rhazya stricta* Decsne (Apocynaceae). African Journal of Microbiology Research, 6(8): 1629-1641.
11. Meda, A., C. E. Lamien, M. Romito, J. Millogo and O. G. Nacoulma (2005). Determination of the total phenolic, flavonoid and proline contents in burkina fasan honey, as well as their radical scavenging activity. Food Chemistry, 91: 571–577.
12. Meelad M. M. (1987). Some Floristic And Chemotaxonomic Studies of the Flowering Plants of Makkah, A thesis submitted for the degree of Doctor of Philosophy of the University of Salford, Department of Biological Sciences University of Salford Salford, Lancs, U.K.

13. Mukhopadhayay S., A. El-Sayed, G. A. Handy, G.A. Cordell (1983). Catharanthus alkaloids XXXVII. 16-Epi-isositsirikine, a monomeric indole alkaloid with antineoplastic activity from *Catharanthus roseus* and *Rhazya stricta*. J. Nat. Prod., 46: 409-413.
14. Norazaidah A. I. Y. and E. K. I. Hainida (2006). Antioxidant activity and phenolic content of raw and blanched Amaranthus species. Food Chemistry, 94: 47-52.
15. Pundarikakshudu K. , C. J. Bhatt and R. K. Patel (2005). Spectrophotometric estimation of total alkaloids from Rauwolfia root powder and formulation. Indian journal of pharmaceutical sciences, 67(5): 614-617.
16. Saeed S. A., R.U. Simjee, F. Mahmood, N. Sultana and Atta-ur-Rahman. (1993). Rhazimine from *Rhazya stricta*: A dual inhibitor of arachidonic acid metabolism and platelet activating factor-induced platelet aggregation. Planta Med., 59: 566–568.
17. Schreier, P., (2005), Chemopreventive compounds in the diet. Dev Ophthalmol, 38: 1-58.
18. Singleton, V. L., R. Orthofer, Lamuela-Raventós, M. Rosa (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin–ciocalteu reagent, 299: 152.
19. Sultana N. and A. Khalid (2010). Phytochemical and enzyme inhibitory studies on indigenous medicinal plant *Rhazya stricta*. Nat. Prod. Res., 24(4): 305-314.
20. Szilaggi, I., E. Mincsovics,, and, G. Kulcsar (1984). Preparation of the homologous components (A_1, A_2 And B) of primycin by thin-layer chromatography. J. Chromatogr. A., 295: 141-151.
21. Verpoorte R. (1998). In Alkaloids: Biochemistry, Ecology and Medicinal Applications, Roberts MF, Wink M (eds). Plenum Press: New York, 397–433.
22. Zaman K. (1990). Studies on the Chemical Constituents of Roots of *Rhazya stricta* Decne. University of Karachi/H.E.J. Res. Inst. Chem., 1-250.