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Design, development and evaluation of polyherbal antioxidant tablet formulation

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ABSTRACT :- Design , development and evaluation of oral polyherbal antioxidant tablet formulation is still a challenge in modern pharmaceutics. The main objective of the present study was to design and develop a polyherbal antioxidant formulation using two different herbs and evaluate their physicochemical study on HPTLC and HPLC, Determination of their antioxidant activity by DPPH method, Tablet formulation prepared by compression methods and evaluate in differents parameter and formulation compared with marketed tablet.

Materials and Methods :-

The polyherbal formulation authenticated herbs were characterized by studying its morphological and pharmacognostic characters. we used potentially active vitis viniferal linn, Pterocarpus marsupium which are the medicinal plants or herbs used for antidiabetic activity.In this work, polyherbal antioxidant formulation were prepared using hydroalcoholic extract of Vitis Vinifera Linn (Seed & Skin) ,P.Marsupium (wood).The Physical, Chemical and Chromatographic evaluation have been studied. The antioxidant activity of the combination of extract was determined using DPPH Method. Tablet formulation prepared by compression method for the treatment of diabetics. It is evaluated by different parameters (weight variation, Hardness, Friability and disintegration time, Formulation compared with marketed formulation).

Result :- The results showed that the combination extract has best antioxidant effect at a dose of 500 mg when it was compared with pterostilbene as the reference standard.We find that evaluation parameters of polyherbal formulation were within acceptable pharmacopoeial limits.

Conclusion :- The results obtained in this research work clearly showed that the combination extract has best antioxidant effect at a dose of 500 mg. Polyherbal antioxidant oral tablet formulation was evaluated and developed as per reference standard.

KEYWORDS :- Polyherbal antioxidant formulation, antidiabetic tablet, Vitis Vinifera Linn, Pterocarpus Marsupium, HPTLC, HPLC.

I. INTRODUCTION :-

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Free radicals are responsible for causing a large number of diseases including diabetics, cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis. Protection against free radicals can be enhanced by various herbal antioxidants. Substantial evidence indicates that polyherbal formulations containing antioxidants are of major importance in disease prevention. There is, however, a growing consensus among scientists that a combination of antioxidants in form polyherbal formulations, rather than single entities, may be more effective over the long term. Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery Antioxidants terminate chain reactions by removing free radical intermediates, and inhibit other oxidation reactions. In recent years, it has been investigated that many plant species are serving as source of antioxidants and received therapeutic significance. The present paper aimed to design, development and evaluation of polyherbal antioxidant tablet formulation containing antioxidant potential of the dried mature fruits of vitis vinifera linn belonging to family vitaceae; Dried heartwood of pterocarpus marsupium belonging to family fabaceae of indian origin was examined. The objective of this work was to perform systematic study for the standardization of grape seed and vijaysar extract. Polyherbal antioxidant formulation were prepared using hydroalcoholic extract of Vitis Vinifera Linn (Seed & Skin) ,P.Marsupium (wood).The Physical, Chemical and Chromatographic evaluation have been studied. The antioxidant activity of the combination of extract was determined using

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DPPH Method. Tablet formulation prepared by compression method for the treatment of diabetics. It is evaluated by different parameters (weight variation, Hardness, Friability and disintegration time, Formulation compared with marketed formulation).Validation of formulation by Linearity and Precision was evaluated by measuring intraday and interday precision. Accuracy was established by performing recovery studies.Prterostibene as a standard, Formulation ,Plant extract and Blank were compared with each other on hplc.methanol was used as the extraction solvent efficiency of the extraction was more with methanol than with other solvents.

II. MATERIALS AND METHODS

Plant materials and authentication

Gallic acid and Pterostilbene using as standard and it is available from Sami labs Limited with 99% Potency. Grapes Seeds Extract (Vitis Vinifera Linn) (G170128) and Vijaysar heartwood Extract (Pterocarpous Marsupium)(H 170081) dried material is available from Sami Labs Limited.

Ingredient

Sodium Carboxy methyl cellulose using as binder. Diluent as mannitol, Sucralose as sweetening agent, Sodium methyl paraben and sodium propyl paraben used as preservative, magnesium stearate used as lubricant.

Method

Standardization of the Extracts

1.Physicochemical Evaluation

The physical evaluation of the combination of all extract was done for following parameters. The results are shown in Table 1.

1. Moisture content
2. pH
3. Water soluble extractive value
4. Alcohol soluble extractive value
5. Total Ash value
6. Acid insoluble ash value
7. Polyphenol assay

2.Chromatographic Evaluation by HPTLC

Conditions were optimized for individual grapes seed extract and Vijaysar extract separately.The mobile phase for HPTLC development was made up of Toluene: EA: Formic acid: Water (8:8:4:1) for grape seed extract and Toluene: EA: Formic acid (10:0.4:0.5) for Vijaysar Extract. The standard solution of Pterostilbene and gallic acid taken 10 ul of each concentration were applied to plates. In stationary phase , an aluminium silica gel 60 F254 plates (20*10cm, 0.2mm thickness)was employed on which the sample and standard (Pterostilbene and gallic acid) were absorbed using a CAMMAG Linomat IV at a pace of 170 nl/S. It was developed in a twin through glass chamber after saturation of mobile phase for 30 min at room temperature upto a length of 80 mm. After drying by an air dryer the TLC Plate was Scanned and digitized. Densitometric Scanning was carried out on a CAMAG TLC Scanner 3 at absorbance 280nm and 307nm. HPTLC Chromatograph and fingerprint for grapes seed extract and vijaysar extract are Shown in Table no 2.

Determination of antioxidant activity by DPPH Method

The free radicals scavenging activity of different extracts was determined by using DPPH assay.the dilutions of 20,40,60,80 and 100 ug/ml were prepared.Each dilution was centrifuged in order to remove any sort of solid matter.the decrease in the absorption of DPPH Solution after the addition of an antioxidant was measured at 307 nm pterostilbene in methanol was used as reference standard. Results is below table no 5

The capability of the formulation to scavenge the DPPH radical was calculated using the formula, percentage inhibition =(AC-AS)/AC*100.

Where, AC is absorbance of control.

AS is the absorbance of sample.

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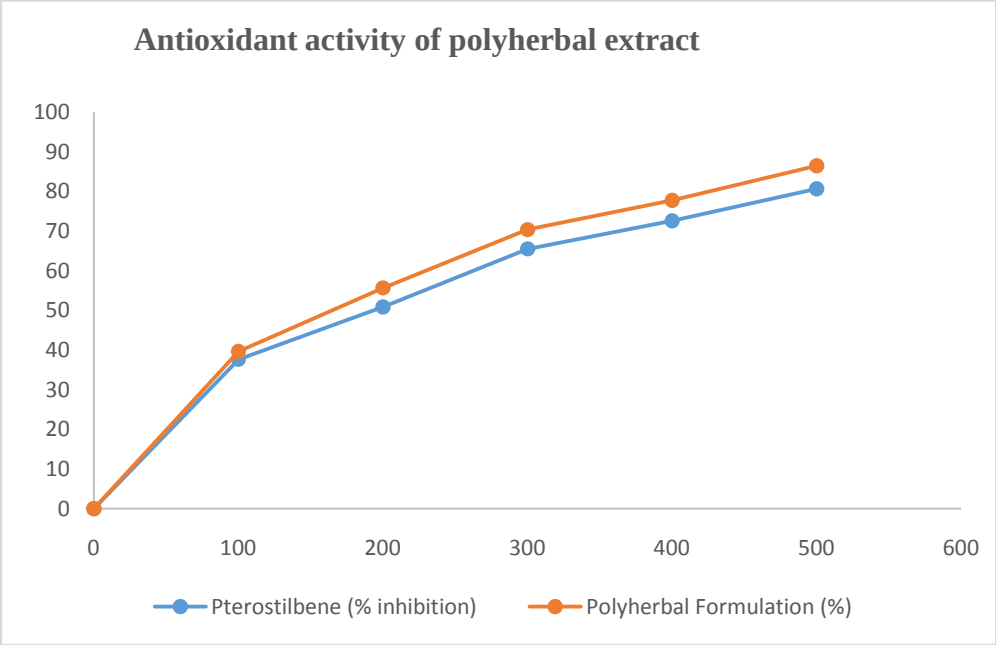
Diagram no 1 :- Antioxidant activity of polyherbal extract



Formulation of polyherbal antioxidant tablet

The hydroalcoholic extract of P.marsupium (wood) and Vitis vinifera (seeds) are the compression method for the treatment of diabetes.

The dried powder extract and other ingredients were mixed uniformly and then mixture was blended and granulated. The granules were then compressed into tablets.The composition of formulation is described in table no 3

SR. NO.	INGREDIENTS	ROLE	MG/TAB	GM/BATCH
1.	Vitis vinifera Extract	Active	200.00	1.00
2.	Pterocarpus marsupium Extract	Active	10.00	0.50
3.	Sodium Carboxy Methyl Cellulose	Binder	199.80	9.99
4.	Mannitol	Diluent	50.00	2.50
5.	Sucralose	Sweetening agent	5.00	0.25
6.	Sodium Methyl paraben	Preservative	0.18	0.01
7.	Sodium Propyl Paraben	Preservative	0.02	0.00
8.	Magnesium Stearate	Lubricant	5.00	0.25
9.	Flavor	Flavoring agent	30.00	1.50
10.	Isopropyl Alcohol	-	q.s	q.s

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Evaluation of Polyherbal antioxidant Tablet
Formulated tablets were evaluated for the following parameters:-
Weight Variation
Hardness
Friability
Uniformity of dispersion
Wetting Volume
Water absorption ration
Dispersion time
Disintegration time
Validation of HPLC method
Linearity and Precision was evaluated by measuring intraday and interday precision. Accuracy was established by performing recovery studies with chromatographic condition in below table no 4.

Column	Phenomenex 5µ C 18(4.6 X 250 mm)
Solvents	Methanol
Flow rate	1.0 mL/min
Column temp	30°C ± 5°C
Sample temp	25° C ± 5°C
Injection volume	10 µl
Detector	PDA at 307 nm
Run time	20 minutes
Retention time	About 12 minute for Pterostilbene

Pterostibene as a standard, Formulation ,Plant extract and Blank were compared with each other on hplc.methanol was used as the extraction solvent efficiency of the extraction was more with methanol than with other solvents.

III. RESULTS AND DISCUSSION

The result obtained from above study indicates the presence of gallic acid andPterostilbene in the PHF.The antioxidant screening done using DPPH method showed a good antioxidant potential as compared to reference standard drug.from the above study,we can conclude that PHF possesses promising antioxidant activity which can be considered as a base for further pharmacological evaluation.

The present paper aimed to design, development and evaluation of polyherbal antioxidant tablet formulation containing antioxidant potential of the dried mature fruits of vitis vinifera linn belonging to family vitaceae; Dried heartwood of pterocarpus marsupium belonging to family fabaceae of indian origin was examined. The objective of this work was to perform systematic study for the standardization of grape seed and vijaysar extract. Polyherbal antioxidant formulation were prepared using hydroalcoholic extract of Vitis Vinifera Linn (Seed & Skin) ,P.Marsupium (wood).The Physicochemical evaluation of individual extract have been studied in Table no 1

Table no 1 Physicochemical evaluation of individual extract

Name of Parameter	Specification of Grape Seed Extract	Results	Specification of Vijaysar Wood Extract	Results
Description	Reddish brown to dark brown powder	Complies	Brown coloured dry powder	Complies
pH	3.0-6.0	3.945	3.0-7.0	3.77
LOD	NMT 5.0% w/w	4.63% w/w	NMT 7.0% w/w	3.01% w/w
Water soluble extractive value	NLT 90.0% w/w	97.66% w/w	NLT 15.0% w/w	56.66% w/w

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Alcohol soluble extractive value	NLT 80%	98.16% w/w	NLT 10.0%	50.16% w/w
Total ash	NMT 2% w/w	0.57% w/w	NMT 15.0% w/w	1.15% w/w
Acid insoluble ash value	NMT 0.5% w/w	0.35% w/w	NMT 5.0% w/w	0.43% w/w
Total Polyphenol content	NLT 30% w/w	42.55% w/w	----------	-------
Total Proanthocyanidins content	NLT 95.0 % w/w and NMT 102.0% w/w	98.65% w/w	----------	-------

Chromatographic evaluation on HPTLC

1. Grape seed Extract have been studied on HPTLC.

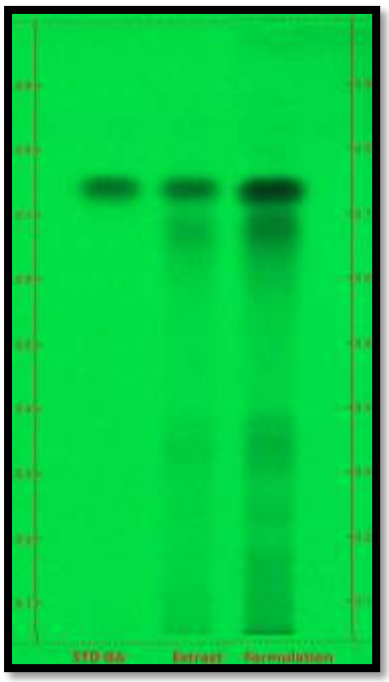

Diagram no :- 2
Grape seed Extract TLC Fingerprinting

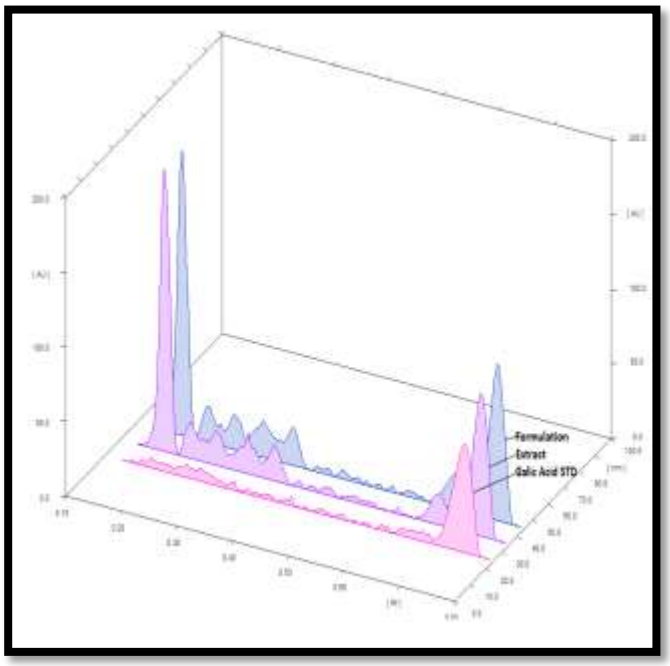

Diagram no :- 3
Grape Seed Extract HPTLC Chromatograph

2. Vijaysar Wood Extract have been studied on HPTLC.

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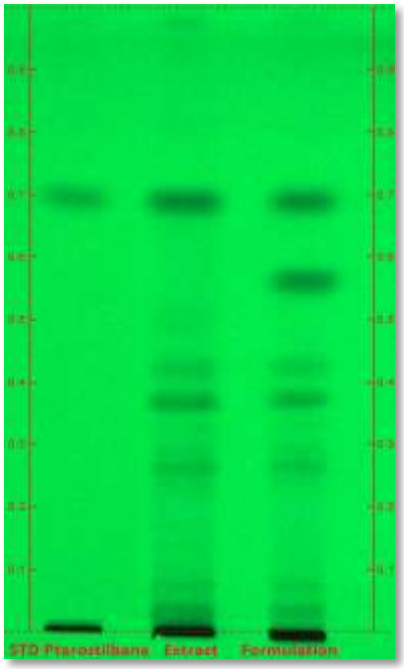

Diagram no :- 4
Vijaysar Extract TLC Fingerprinting

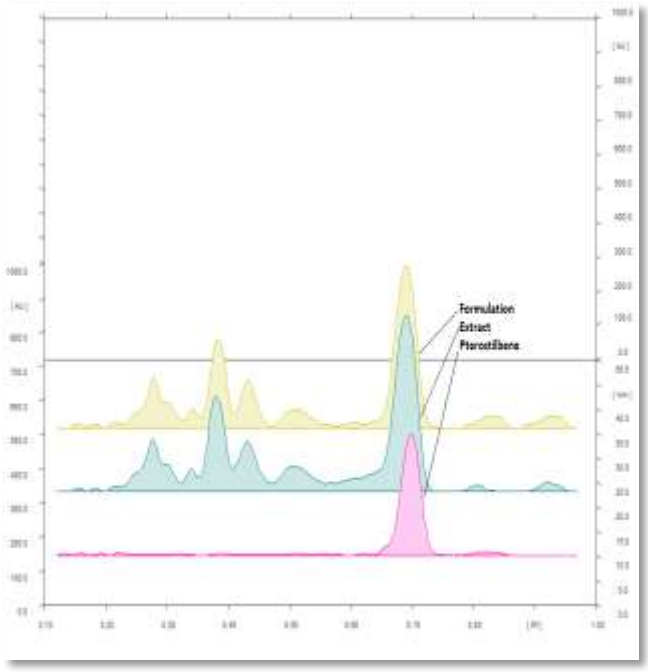

Diagram no :- 5
Vijaysar Extract HPTLC Chromatograph

Table no 5 :- DPPH Antioxidant activity of polyherbal formulation with reference to Pterostilbene

Sr. No	Concentration (100 ug/ml)	Pterostilbene (% inhibition)	Polyherbal Formulation (%)
1	100	37.66	39.65
2	200	50.87	55.68
3	300	65.52	70.39
4	400	72.56	77.76
5	500	80.66	86.5

Evaluation of Polyherbal Tablet

Table no 6 :- Evaluation of polyherbal tablet

Parameter	**Results**
Appearance	Oval shape maroon coloured white spot tablet
Thickness	5.76mm
Weight Variation	501mg
Hardness	6.33kg/cm^2
Friability	0.249
Uniformity of Dispersion	Passes test
Wetting Volume	8.16
Wetting Time	40sec
Water Absorption Ratio	92.04%
Dispersion Time	113.33
Disintegration Time	56.33

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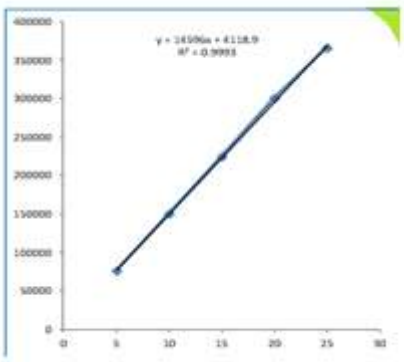
HPLC Validation of Formulation with reference standard

Table no 7 :- Linearity of Pterostilbene for HPLC

Conc. of Pterostilbene (ppm)	Average Peak Area of Pterostilbene
5	75485
10	14986
15	22457
20	30012
25	36526



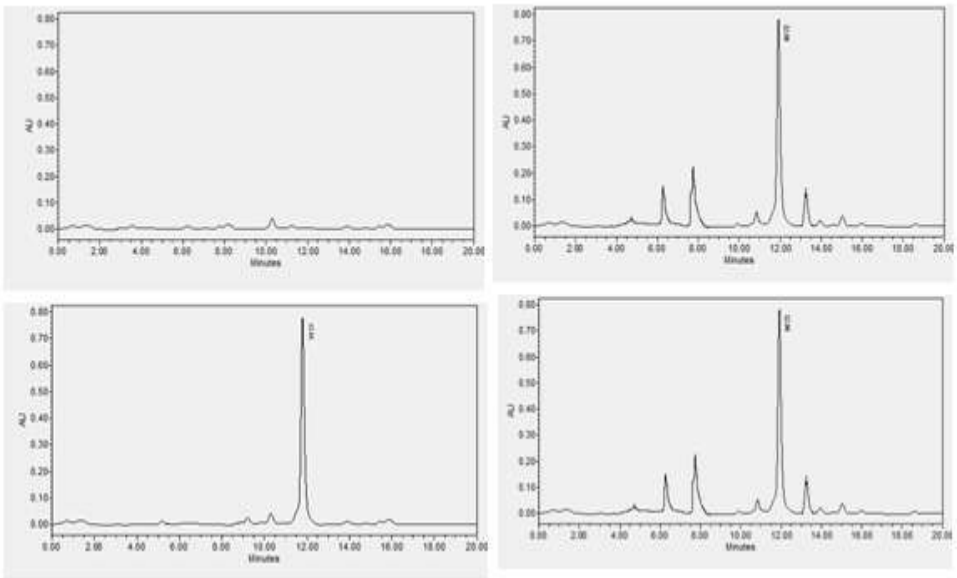


Diagram no :- 6 HPLC Chromatogram

Table no :- 8

Sr. No.	Sample name	RT	Tailing Effect	Purity
1	Pterostilbene STD	12 minutes	NO	Passed
2	Blank	No band is observed	NO	Passed
3	Extract	About 12 minutes	NO	Passed
4	Formulation	About 12 minutes	NO	Passed

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5	Placebo Solution	No band isobserved	NO	Passed

Table no 9 :- Precision and recovery of extract and formulation

PARAMETERS		EXTRACT	FORMULATION
Intra-day Precision (% RSD)		5.01	4.99
Inter-day Precision (% RSD)		5.02	5.00
% Recovery	80%	98.62	98.43
	100%	98.99	98.77
	120%	99.83	98.84

Table no 10 :- Robustness parameter for HPLC

ROBUSTNESS PARAMETER		% RSD
Column temperature (°C)	28	0.88
	30	1.45
	32	1.69
Flow rate (ml/Minute)	0.9	1.77
	1	1.40
	1.1	1.01
Wave length (nm)	340	1.82
	345	1.18
	350	1.23

REFERENCES

[1]. Partap, S., 2012. A Review on Herbal Antioxidants. Journal of Pharmacognosy and Phytochemistry,1(4).

[2]. Madhumita, G. and Rajini, G., An alliance between Brand Awareness & Purchase Decisions for facial cream brands among Women Professionals–A Path Analysis Approach.

[3]. Percival, M., 1999. Understanding the natural management of pain and inflammation. Clin. Nutr. Insights, 4, pp.1-5.

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[4]. Sisein, E.A., 2014. Biochemistry of free radicals and antioxidants. Scholars Academic Journal of Biosciences, 2(2), pp.110-118.
[5]. Nassiri-Asl, M. and Hosseinzadeh, H., 2016. Review of the pharmacological effects of Vitis vinifera (grape) and its bioactive constituents: an update. Phytotherapy Research, 30(9), pp.1392-1403.
[6]. Halliwell, B., Zentella, A., Gomez, E.O. and Kershenobich, D., 1997. Antioxidants and human disease: a general introduction. Nutrition reviews,55(1), p.S44.
[7]. Kumar, S., 2014. The importance of antioxidant and their role in pharmaceutica science-a review.Asian J. Res. Chem. Pharm. Sci, 1(1), pp.27-44.
[8]. Panchawat, S., Rathore, K.S. and Sisodia, S.S., 2010. A review on herbal antioxidants. Intern J Pharm Tech Res, 2, pp.232-239.
[9]. Lobo, V., Patil, A., Phatak, A. and Chandra, N., 2010. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy reviews, 4(8), p.118.
[10]. Mandic, A.I., Đilas, S.M., Ćetković, G.S., Čanadanović-Brunet, J.M. and Tumbas, V.T., 2008. Polyphenolic composition and antioxidant activities of grape seed extract. International Journal of Food Properties, 11(4), pp.713-726.
[11]. Ma, Z.F. and Zhang, H., 2017. Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. Antioxidants, 6(3), p.71.
[12]. Kokate CK, Purohit AP, Gokhale SB. Text Book of Pharmacognosy. 18th ed. Pune: Nirali Prakashan; 2002.
[13]. Lala PK. Practical Pharmacognosy. 1st ed. Calcutta: Lina; 1981.
[14]. Harborne JB. Phytochemical Methods-a Guide to Modern Techniques of Plant Analysis. Berlin: Springer Verlag; 2005.
[15]. Kaul S, Dewivedi Indigenous ayurvedic knowledge of some species in the treatment of human disease and disorders. International Journal of Pharma Life Sciences 2010: 1 : 44–49
[16]. Kumar T, Chandrashekhar KS ,Bauhinia purpurea Linn: a review of its Ethanobotany, phytochemical and pharmacological profile. Research Jounal of Medicinal Plant 5:420–431
[17]. Kumar CH, Dr. Kumar JNS, Ishaq BM, Rani GU, Prkash KV. Antidiabetic activity of a polyherbal preparation, Pharmacologyonline 2010; 2: 780-787.
[18]. Nishant Pathak, Gyanendra Kumar and Rakesh Chandra Chaurasia. Evaluation of anti-diabetic activity of commercially available extracts of Phyllanthus emblica in Streptozocin induced diabetic rats. Int J Pharm Bio Sci 2016 Oct; 7(4): 139 - 145.
[19]. Alagar raja M, Shailaja V, David Banji, Rao K.N.V, SelvaKumar.D, Evaluation of Standardiasation parameters, Pharamacogonstic Study, Preliminary Phytochemical Screening and In vitro Ant diabetic ability of Emblica officinalis fruits as per WHO guideline, Journal of Pharmacognostical and Phytochemical 2014; 3(4): 21-28.
[20]. Aji Abraham, Lizzy Mathew and Sarala Samuel, Pharmacognostical Studies of the fruits of Terminalia Belerica (Gaertn.) Roscb, journal of Pharmacognosy and phytochemistry 2014; 3(2): 45-52.
[21]. Nidhi Khemaka, Harisha C R, Shukla V J, Galib R, Patgiri, Prajapati P K, Preliminary Pharmacognostical and Phytochemical Evaluation of Prashata and Aprashsata Haritaki (Terminalia Chebula Retz), International Journal of Ayurvedic medicine 2014; 5(3): 257-268
[22]. Kokate CK, Purohit AP, Gokahle SB. Pharmacognosy. 24thed.Vallabh Prakashan, Pune. 2003; pp.108-109.
[23]. Gurdeep R, Chatwal, Sham K Anand, Instrumental methods of chemical analysis,Mumbai. Himalaya Publication House 2005; 2.272-2.302
[24]. Beckett AH, Stenlake JB, Practical Pharmaceutical chemistry 2nd Ed, New Delhi: CBS publishers, 2001,115-126.
[25]. Kasture A V, Mahadik K R, Wadokar S G, More H N. Chromatography, Pharmaceutical analysis. 15th edition. Pune. Nirali Publication. 2006