



# AMERICAN JOURNAL OF PHARMTECH RESEARCH

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## Pharmacognostical and Preliminary Phytochemical Studies of *Argyrea speciosa* Leaves

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### ABSTRACT

The present work on the leaves of *Argyrea speciosa* Sweet (Convolvulaceae) has led to the pharmacognostical and phytochemical parameters. Macroscopical and microscopical characters, micrometry, physio-chemical constants, quantitative microscopy parameters, extractive values, carried out. The study also deals with the phytochemical screening of with various extracts. The total phenolic (TPC) and Total flavonoids contents (TFC) in leaves of *Argyrea speciosa* were studied with the aim of drawing the standards. The leaf is heart shaped upto 12.0-17.1-20.3 X 11.9-15.45-23.7 cm across, back with white shiny hairs on the lower surface, glabrous above, tomentose beneath and long stalked. Microscopically the leaves show cuticle, lignified xylem (3.48-5.10-7.43 $\mu$ ), phloem (1.45-2.85-4.25 $\mu$ ), starch grains, upper and lower epidermal cells were identified. Unicellular pointed tip trichomes are numerous and present on dorsal side abundantly. The palisade cells are rectangular in nature up to 10.32-13.41-16.50X1.93-2.77-3.57 $\mu$ . The preliminary investigations showed that the moderate presence of terpenes, flavonoids, steroids, phenols and tannins. The TPC found to be, 173.55+0.017 mg (gallic acid equivalent/g) and TFC 134.07+0.123 mg (quercetin equivalent/g). In addition, total tannin content (TTC) determined by back titration with potassium permanganate and, was found as 087.00+0.17mg (tannin equivalent/g). In study TPC, TFC and TTC are significant and prove that, leaves are rich in estimated phytoconstituents and may have pharmacological importance.

**Keywords:** *Argyrea speciosa*, Macroscopy, Microscopy, Phenolic, Flavonoid, Tannin.

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Received 01 September 2018, Accepted 22 September 2018

Please cite this article as: Nagare B *et al.*, Pharmacognostical and Preliminary Phytochemical Studies of *Argyrea Speciosa* Leaves . American Journal of PharmTech Research 2018.

## INTRODUCTION

*Argyrea speciosa* (Burm. F) family: Convolvulaceae, commonly known as 'Vidhara' is a popular Indian medicinal plant, which has long been used as a 'rasayana' drug in traditional Ayurvedic medicine. This plant is pharmacologically studied for hepatoprotective, anti-oxidant, anti-inflammatory, wound healing activity and immunomodulatory activity. A wide range of phytoconstituents isolated from this plant like 9-keto-octadec-15-enoic acid, 6-methoxy coumarin-7-O- $\beta$ -glucopyranoside, quercetin and kaempferol<sup>1,2,3</sup>. This study intended to establish, macroscopical, microscopical, chemo-microscopical, quantitative evaluation of the powdered and fresh leaves of the plant to be used as diagnostic features in identification, evaluation and monograph preparation of the plant.

## MATERIALS AND METHOD

### Plant Collection and Identification

Whole plant of *Argyrea speciosa* was collected from Gorhewadi (Nashik road), Maharashtra, India in the month of Aug-Sep 2017. Plant sample was identified and authenticated by Prof. Rajesh T. Wankhede, Dept. of Dravygana, S.M.B.T. Ayurvedic College and Hospital, Nashik, India. Voucher specimen of the plant material has been deposited at Institute level (SMBTIODP/HERB/04-2018).

### Chemicals and Instruments

Compound microscope, simple microscope, glass slides, cover slips, watch glass and other common glassware were the basic apparatus and instruments used for the study. Photomicroscope provided with OLYMPUS Magnus software with Magcam DC 5MP camera. Some crystals, starch grains and lignified cell slides were taken under projection microscope. Solvents viz. petroleum ether, chloroform, methanol, ethanol and reagents viz. phloroglucinol, glycerin, HCl, Iodine and potassium hydroxide were procured from Loba Chemicals, Mumbai, India.

### Preparation of Plant Material

Fresh mature leaf are store in formalin solution and powder of leaf *Argyrea speciosa* was prepared by passing through sieve # 44, and kept in air tight polythene bags for further study<sup>4</sup>.

### Extraction

Methanol used for extraction, 100 g of the powder was extracted using Soxhlet's apparatus for 6 hrs. The extract dried and kept in container for further study.

### Macroscopical Examinations

For morphological observations, fresh young leaves were used<sup>5</sup>. The macro morphological features of the plant parts (leaves) were observed under magnifying lens and simple microscope<sup>6</sup>.

### **Microscopical Examinations**

Fresh leaves of the plant were studied transversely and longitudinally, using surface preparations and sections. The different parts of leaf like lamina and midrib were studied according to the methods of Brain and Turner<sup>7</sup>. For the microscopical studies, cross sections were prepared and stained as per the procedure of Khandelwal KR<sup>8</sup>. Quantitative evaluations and quantitative-leaf microscopy were also carried out as outlined by Wallis TE<sup>9,10</sup>. Chemo-microscopical examinations were also carried out, following thorough clearing of the powdered leaves with potassium hydroxide solution and a subsequent mounting with dilute glycerol on a microscope slide, and tested with various detecting reagents. Various chemical constituents were identified in accordance with Kokate CK<sup>11</sup>.

### **Preliminary Phytochemicals Investigation**

The investigation was carried out by using standard procedures<sup>12</sup>.

### **Quantitative Evaluations of the Crude Drug**

Moisture content of the powdered leaves determined based on the loss of drying method<sup>13</sup>. The ash values (Total ash, acid insoluble ash and water-soluble ash) were determined, to find out about the physiological state and level of extraneous matter. Total ash of the drug was subjected for testing different inorganic constituents<sup>14,15</sup>. Extractive values (ether, methanol, chloroform, alcohol and water) were determined according to the official methods prescribed in Ayurvedic pharmacopoeia<sup>16</sup>. Fluorescence analysis of powdered leaf was done by standard method of Chase and Pratt<sup>17</sup>.

### **Determination of TPC**

The TPC of the *Argyrea speciosa* leaf methanol extracts was determined using the Folin-Ciocalteu reagent. The diluted methanolic extract (0.5 ml of 1:10 g mL<sup>-1</sup>) or gallic acid (standard phenol compound) was mixed with Folin - Ciocalteu reagent (5 ml, 1:10 diluted with distilled water), and aqueous sodium carbonate (4 ml, 1 M). The reaction mixture was kept in dark at ambient conditions for 0.30 h to complete the reaction. The absorbance at 765 nm was measured<sup>18</sup>. Gallic acid was used as standard and the results were expressed as mg gallic acid equivalent/g *Argyrea speciosa* leaf.

### **Determination of TFC**

Total flavonoid content was determined using aluminum chloride (AlCl<sub>3</sub>) according to a known method, using quercetin as a standard. Plant extract (0.5 ml of 1:10 g mL<sup>-1</sup>) in methanol were

separately mixed with 1.5 ml of methanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100  $\mu\text{g mL}^{-1}$  in methanol<sup>19</sup>. The results were expressed as mg quercetin equivalent/g *Argyreaia speciosa* leaf .

### Estimation of TTC

TTC was determined by back titration with potassium permanganate solution. Accurately weighed 0.5 g (W) of plant material boiled with 20 ml water for 15 minutes and then filtered. The step was performed four times; final volume was made to 100 ml. 10ml filtrate and 10 ml indigo carmine solution was added. Diluted this mixture to 100 ml with water. Lastly, back titrated against 0.1 N  $\text{KMNO}_4$  still the golden yellow color is obtained. Obtained volume (T2) subtracted from initial volume (T1) of 0.1 N  $\text{KMNO}_4$  and used in percentage calculation. TTC calculated by applying the following factor; 1 ml 0.1 N  $\text{KMNO}_4 \approx 0.00416$  g tannins and percentage quantity of total tannins=  $[(T2-T1) \times \text{actual normality} \times 0.004157 \times 1000]/W \times 0.1$ <sup>20</sup>.

## RESULTS AND DISCUSSION

### Macroscopic Examination

The macro-morphological characteristic of the leaves of *Argyreaia speciosa* identified were acute apex, crenate margin, symmetric base, reticulate venation and hairy to softly pubescent shape. Leaves surface thick, with dimension 12.00-17.1-20.30 x 11.9-15.45-23.70 cm. Color is pale green to green; taste is slightly mucilaginous, odor is none. The plant shows covering trichomes more on the lower surface compare to upper surface of the leaves (Figure 1).



**Figure 1: Morphology of *Argyreaia speciosa* Leaf**

### Microscopic Examination

Transverse section of leaf (Figure 2), it is a dorsiventral leaf. Following tissues are present in **Lamina**, The Upper epidermal cells are compactly arranged with no intercellular spaces except

stomata. Epidermis is made up of single layer of cell. They are straight walled polygonal present at upper and lower side. Mucilage is detected in epidermis. Mesophyll is differentiated into palisade and spongy parenchyma. Palisade single layered elongated compactly arranged, narrow columnar cells with beaded anticlinal walls. Uniseriate layer has been continuous over the midrib region. Palisade cells were filled with chlorophyll. Spongy parenchyma present with 3-4 layered, loosely arranged with intracellular space and varying size and shape. In it Starch grain and calcium oxalate crystals were present. Chlorophyll is present in epidermal cells, numerous paracytic stomata, and unicellular clothing trichomes were observed on the both epidermis.



**Figure 2: Transverse section of *Argyreia speciosa* Leaf.**

*Ct*-Cuticle; *uEp*-Upper Epidermis; *Pl*-Palisade cells; *lEp*-Lower Epidermis *uCol*- Upper collenchyma; *lCol*- Lower collenchyma; *ngTrc*- Non glandular Trichomes; *Xy*- Xylem; *Ph*- Phloem;

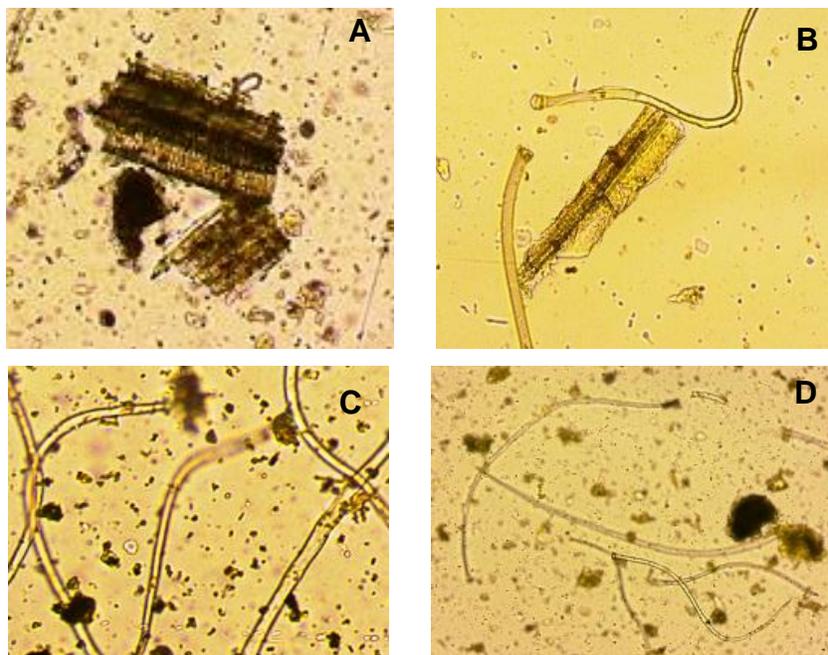
**Midrib**, present both surface with different degree of concavity. Dorsal surface is more convex than ventral surface. Lower palisade is replaced by patch of 2-4 layered collenchymatous cells, which is about  $4.82\mu$  in diameter. Collateral vascular bundle is prominent, occupying the central portion of the midrib. Xylem vessels are covered by xylem fibers. Xylem parenchyma is made up from semirectangular, lignified cells. The phloem is non-lignified and collenchymatous in nature. The micrometric analysis is tabulated in Table 1.

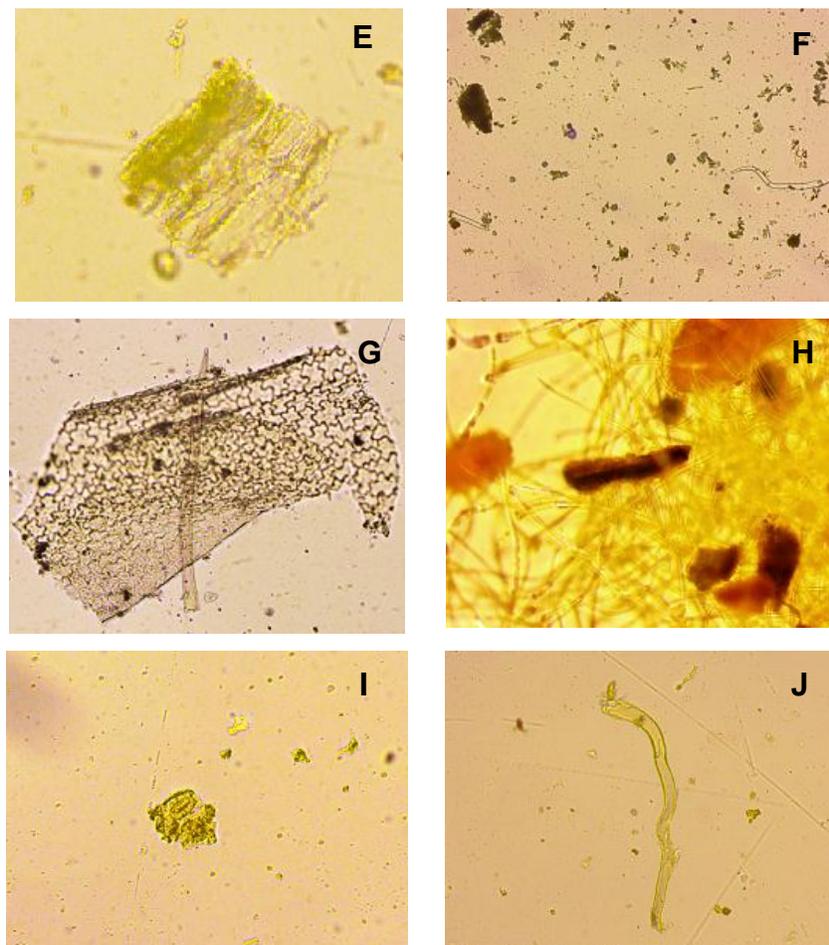
**Table 1: Micrometry of Cellular Elements**

Cellular elements /cell content	Measurement ( $\mu$ m)
Upper epidermal cell	Length: 02.78-07.14-11.80; Width: 01.65-02.76-03.66
Lower epidermal cell	Length: 01.08-02.50-04.15; Width: 01.10-01.57-02.04
Palisade cell	Length: 10.32-13.41-16.50; Width: 01.93-02.77-03.57
Upper collenchymatous cell	00.80-01.55-02.93
Lower collenchymatous cell	01.93-04.82-05.50
Parechymatous cell	07.05-09.60-15.25
Phloem cell	01.45-02.85-04.25
Xylem vessels	03.48-05.10-07.43

### Powder Characteristic

The leaf powder have fluffy nature, greenish color with mucilaginous taste. No greasy stain was observed when powder press between Whatmann's filter paper No. 40 indicates absence of fixed oil. On microscopically examination, the powder showed





**Figure 3: Powder study of *Argyreia speciosa* Leaf**

*A- Xylem Vessel-Scleriform thickening; B- Xylem Tissue; C- Calcium Oxalate Crystal; D- Trichomes; E- Mesophyll Tissue; F- Starch Grain- Stained by Iodine solution; G- Epidermal Cell; H- Lignified Tissues; I- Stoma; J- Xylem Fibre.*

Trichomes - Non-glandular unicellular trichomes with pointed tip observed.

Xylem fibers - Scaliform with ladder like thickening.

Mesophylls – Fragments of leaf showing spongy parenchyma cells.

Starch grains are simple granules, spherical or irregularly ovoid and less prominent striations.

Large lignified cell entire or scattered forms.

### **Quantitative Microscopical Parameters of Leaves**

Pertaining to the stomatal index, stomatal number, vein islet number and Vein-termination number data given in Table 2.

**Table 2: Quantitative Parameters of Leaves**

Leaf Constants	Size ( $\mu\text{m}$ )
Diameter of stomata	Upper surface; 39.0-41.0-43.0 Lower surface; 36.0-38.0-40.0
Stomatal number	Upper surface; 50.0-57.0-64.0 Lower surface; 26.0-27.0-28.0
Stomatal index	Upper surface; 4.8/Sqmm Lower surface; 17/Sqmm
Vein-islet number	12.05/Sqmm
Vein-termination number	14.65/Sqmm

### Chemo-microscopical Examination

The counter idea about presence of phyto constituents is obtained through this study like phenolic compound in palisade as indicated by brownish black stain on ferric chloride solution treatment (Table 3).

**Table 3: Chemo-micro chemical Tests of *Argyrea speciosa* Leaf**

Reagent	Color	Test for	Histological zone
Phloroglucinol + Hcl	Pink	Lignin	Vascular bundle
Weak iodine solution	Blue	Starch	Mesophyll region
Ferric chloride solution	Brownish black	Phenolics	In palisade cell region
Libermann-Burchardt reagent	Greenish	Steroids	Mesophyll region
Million's reagent	Blue	Proteins	Vascular bundle

### Preliminary Phytochemicals Investigation

Revealed the presence of primary and secondary metabolites as carbohydrates, mucilage, tannins, Terpenoid, Glycosides, Alkaloids and Phytosteroids (Table 4).

**Table 4: Preliminary Phytochemicals Investigation**

Phyto constituents	Pet. ether	Chloroform	Methanol	Ethanol	Water
Carbohydrates	-	-	-	+	+
Steroids	+	-	-	-	-
Alkaloids	-	+	-	-	-
Glycosides	-	+	-	+	-
Reducing sugar	-	-	-	-	+
Phenolic	-	-	+	+	-
Tannins	-	-	+	+	-
Proteins	-	-	-	-	-
Amino acid	-	-	-	-	-
Mucilage	-	-	-	-	+

‘+’ presence; ‘-’ absence

### Quantitative evaluations of the crude drug

The moisture content seems to be lower than necessary to support the growth of microbes to bring any change in the composition of the drugs. Physical constant as ash value of the drug gives an

idea of the earthy matter or the inorganic composition and other impurities present along with the drug. Extractive values are useful for the determination of exhausted or adulterated drugs. The results of the physical constants of the drug powder are given in Table 5.

**Table 5: Quantitative Evaluations**

Parameter	Values % (w/w)* $\pm$ SD
Total ash	14.90 $\pm$ 0.13
Acid insoluble ash	03.10 $\pm$ 0.21
Water soluble ash	05.11 $\pm$ 0.18
Moisture content (dry weight basis)	02.05 $\pm$ 0.16
<b>Extractives;</b>	
Petroleum ether soluble	02.03 $\pm$ 0.31
Ethanol soluble	15.10 $\pm$ 0.03
Water soluble	18.11 $\pm$ 0.24

The qualitative analysis of ash indicated presence of calcium, Potassium, magnesium, sodium and phosphates (Table 6).

**Table 6: Qualitative Analysis of Elements Present in Total Ash**

Element	Inference
Aluminum	--
Calcium	--
Potassium	++
Magnesium	+-
Sodium	++
Iron	++
Zinc	--
Antimony	--
Chlorides	++
Sulphate	--
Phosphates	++
Carbonates	--

'++' presence; '--' absence

#### Determination of TPC, TFC and Estimation of TTC

The leaf of *Argyrea speciosa* methanol extract have the abundant quantity of phenols which expressed in term of gallic acid (the standard curve equation:  $Y = 0.095X + 0.029$ ,  $r^2 = 0.996$ ), flavonoids is expressed in term of quercetin (the standard curve equation:  $Y = 0.217X + 0.388$ ,  $r^2 = 0.991$ ) and tannins (Table 7).

**Table 7: TPC, TFC and TTC of *Argyrea speciosa* Leaf on Dry Weight Basis**

Parameter	Values mg/g	Equivalent/g
TPC	173.55 $\pm$ 0.017	Gallic acid
TFC	134.07 $\pm$ 0.123	Quercetin
TTC	087.00 $\pm$ 0.170	Tannins

## CONCLUSION

The exhausted literature survey conclude before the present work start there is no pharmacognostical study on record of this much valued traditional drug. Therefore present work was taken up with a view to lay down standards which could be useful to find the authenticity of this traditional medicinal plant. In other words, the pharmacognostical features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations at Herbal industrial level in the coming days.

Phenolic, flavonoid and tannins compounds are a class of antioxidant agents which act as free radical terminators and their bioactivities may be related to their abilities to chelate metals, inhibit lipooxygenase and scavenge free radicals<sup>21,22</sup>. Free radicals are often generated as byproducts of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases<sup>23</sup>. The *Argyreia speciosa* leaf may have high scavenging property due to hydroxyl groups existing in the phenolic compounds. The total flavonoid contents indicate there might be a chance to search new flavonoids in leaf of *Argyreia speciosa*.

## ACKNOWLEDGEMENT

The author(s) are thankful to management of S.M.B.T. Sevabhavi Trust, Dhamangaon (Nashik; India) for providing facilities.

## REFERENCES

1. Kirtikar KR, Basu BD. Indian Medicinal Plants, Vol.III, Dehradun, India: International Book Distributors; 1999; 2352-59.
2. Annonymous. The wealth of India (Raw materials), Vol I:A, New Delhi: Council of Industrial and Scientific Research; 2010; 418-19.
3. Nadkarni KM. Indian Materia Medica, Vol I, Mumbai: Popular Prakashan; 2000; 136-37.
4. Rangari VD. Pharmacognosy and Photochemistry, Part-I, 1<sup>st</sup> ed., Nashik: Career Publication; 2002; 368-94.
5. Trease GE, Evans MC. Textbook of Pharmacognosy, London: Balliere - Tindall,; 1983: 253-88, 519 - 21.
6. Tyler V, Brady L, Robbers J. Pharmacognosy, India: K. M. Varghese Company; 1977: 103-41.
7. Brain KR, Turner TD. The practical Evaluation of Phytopharmaceuticals, Bristol: Wright-Scientehnica; 1975: 81 -6.

8. Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, Pune: Nirali Prakashan; 2005: 30-49.
9. Wallis TE. Practical Pharmacognosy, London: J. and A. Churchill Ltd.; 1953: 139.
10. David V WB. Morphology of Vascular Plants, London: The Macmillan; New York Ltd.; 1971: 499.
11. Kokate CK. Handbook of Practical Pharmacognosy, New Delhi: Vallabh Prakashan; 1994: 58-136
12. Harborne JB. Phytochemical Methods, London: Chapman and Hall; 1984: 88, 203.
13. Anonymous. WHO, Geneva Quality Control methods for medicinal plant material, New Delhi: A.I.T.B.S. Publishers and Distributors; 2002: 31.
14. Government of India. Ministry of Health and family Welfare. Indian Pharmacopoeia. Vol II, The controller of Publication, New Delhi; 1996: 390, A-100
15. Anonymous. Indian Herbal Pharmacopoeia, Mumbai: Indian Drug Manufacturer's Association; 2002: 1-11.
16. Government of India. Ministry of Health and family Welfare. The Ayurvedic Pharmacopoeia of India. Part-I, Vol I, The Controller of Publication, New Delhi; 1985: 24-8, 143.
17. Chase CR and Pratt RJ. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J. Am. Pharmacol. Asso. 1949; 38: 32.
18. Barku VYA, Opoku-Boahen Y, Owusu-Ansah E, Mensah EF. Antioxidant activity and the estimation of total phenolic and flavonoid contents of the root extract of *Amaranthus spinosus*. Asian Journal of Plant Science and Research, 2013; 3(1):69-74.
19. Sahu R, Saxena J. Screening of Total Phenolic and Flavonoid Content in Conventional and Non-Conventional Species of Curcuma. Journal of Pharmacognosy and Phytochemistry 2013; 2 (1):176-179.
20. Tatiya AU, Saluja AK. Evaluation of Phytochemical standards and In Vitro Antioxidant Activity of tannins rich fraction of Stem Bark of *Bridelia retusa* (Li). International J PharmTech Res 2010; 2(1): 649-655.
21. Ushir YV. Estimation of secondary metabolites in different tea and coffee brands from Indian market. Int J Pharm & Life Sci 2011; 2(3):599-600.

22. Roya K, Fatemeh G. Screening of total phenol and flavonoid content, antioxidant and antibacterial activities of the methanolic extracts of three *Silene* species from Iran., Intl J Agri Crop Sci 2013; 5 (3): 305-312.
23. Mannan H. Total phenolic, flavonoid, tannin content and antioxidant power of some Iranian pomegranate flower cultivars (*Punica granatum* L.). American J Plant Sci 2013; 4: 1815-1820.

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